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CARDIO-VASCULAR REACTION IN THE VALSALVA EXPERIMENT AND IN LIFTING WITH A NOTE ON PARTURITION[1]

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From the Physiological Laboratory of the University of Wisconsin

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INTRODUCTION

The object of the following experiments was to determine the immediate effect upon the heart rate and blood pressures of exercises of strain. By "exercises of strain" is meant those muscular efforts which are performed with the glottis closed and which tend to compress the chest, thereby causing a rise in intrathoracic pressure. The two forms of strain studied were the Valsalva experiment and lifting.

The importance of the subject may be realized when we remember that the conditions which obtain during lifting and the Valsalva exper-

[1] A preliminary report of this work was made at the annual meeting of the American Physiological Society and was published in this Journal (2).

[2] It has seemed to me no more than just to place the name of Doctor Hodges where it stands. I am, however, reluctant to make him responsible for statements, perhaps erroneous, which are not his and in regard to which I have not consulted him owing to his absence in China. Consequently I offer this word of explanation.

In 1914–1915 Doctor Hodges and I worked upon the Valsalva experiment and our results appeared in his Bachelor's thesis (3) and in a preliminary report presented by me (2). Subsequently I continued and amplified this work considerably and entirely rearranged such parts of the thesis and preliminary report as I wished to embody in the final communication.

I think it would have been absurdly awkward to have presented separately our work and mine.—P. M. D.

iment are very similar to those which occur in difficult defecation, parturition and severe cough.[3]

In this article will be found a consideration *a*, of the Valsalva experiment; *b*, of lifting; *c*, of certain modifications which may be applied to these two maneuvers; and lastly, *d*, of an interesting observation on a rabbit during labor reflexly induced.

THE VALSALVA EXPERIMENT

The Valsalva experiment consists in a forced expiratory movement with the glottis closed. The effectiveness of the expiratory effort may be enhanced by taking a deep breath just before the forced expiration has begun and also by raising the knees and pressing the thighs firmly against the abdomen, so as to support the abdominal walls during the expiratory movement. Usually the effort was continued as long as possible, that is, until the respiratory distress became excessively disagreeable.

A discussion of the phenomena observed during the Valsalva experiment falls naturally into three parts which concern the blood pressure, the pulse rate and the pulse form respectively. This separation is not only on the basis of subject matter but also on that of method of experimentation, the sphygmomanometer, the string galvanometer and the sphygmograph being the instruments respectively employed in each part of this study. The sphygmomanometer was that of Erlanger which was connected with a tank of compressed air by means of which the cuff could be inflated more rapidly than with the bulb as ordinarily employed. The graphic method was supplemented by the auscultatory, use being made of whichever method seemed the more suitable to the special occasion. The sphygmograph was that of Dudgeon, modified as suggested by Lewis (4) by the substitution of freely hanging weights for the straps which ordinarily secure the instrument to the wrist.

Blood pressure (systolic)

1. Primary rise (of McCurdy) (6). The cuff was strapped about the subject's arm, with the stethoscope below, and the normal systolic pressure determined by the auscultatory method. Air was then allowed to enter the cuff rapidly until the mercury stood at 180 mm.;

[3] The history of the Valsalva experiment has seemed so interesting to one of us (D) that he purposes to present it in a separate communication.

at this point the subject was instructed to fill his lungs and strain with closed glottis. If the resulting rise in blood pressure surpassed 180, it caused the pulse to "break through" beneath the cuff, producing a sound which the observer could hear with the stethoscope. The time elapsing between the beginning of the effort and the appearance of this sound, together with the total duration of the effort, were recorded by means of a stop watch. In this way it was possible to determine at what stage of the effort the blood pressure reached 180 mm. Hg., and, by repeating the procedure with increasing pressures in the cuff, it was possible to find the maximum height to which the blood pressure rose, that is, the pressure in the cuff beyond which the pulse failed to "break through."

The following protocols of April 1, 1915, are typical:

Series I: subject Dawson; normal systolic pressure 122 (two concordant observations); with pressure in cuff 180 mm., one pulse beat was heard a few seconds after the beginning of the expiratory effort; with pressure in cuff 190 mm. several pulse beats were heard; when a second trial was made with pressure in cuff 180 mm., several pulse beats were heard; at 190 mm., none.

Series II: Subject P. C. Hodges; normal systolic pressure 112 (average of several closely agreeing observations); with pressure in cuff 130 mm., two pulse beats were heard; at 140 mm., one beat; at 156, six; 172, three; 180, three; 190, none.

Six subjects thus examined gave primary blood pressure rises of from 62 to 90 mm. Hg., the greatest total pressures being between 180 and 200. The average time elapsing between the beginning of the effort and the height of the rise was about three seconds, the duration of the rise being very short.

2. Fall (of Valsalva and Weber) (7). Following the primary rise of systolic pressure there is a very considerable fall due without doubt to the decreased inflow of blood to the heart resulting from the high intrathoracic pressure. Two methods were employed in estimating the extent of this fall. Though neither was such as to establish precisely the quantitative value of the fall, both were highly suggestive.

First method. Five subjects were examined, but since the results coincided very closely, a description of the one case will serve for all. The cuff and stethoscope were adjusted as usual, and the pressure raised to 60 mm. At this point the beating of the pulse could be plainly heard. Eight seconds after the beginning of the effort the sound failed, and could not be heard again, though the pressure in the cuff was quickly raised and lowered between 30 and 150 mm. through-

out the rest of the exercise. With expiration the sound promptly reappeared, and the pressure soon returned to normal. The fact that the sound failed with the pressure in the cuff at 60 mm. points to the assumption that either both systolic and diastolic pressures were above 60 mm. during that part of the exercise, or that they had both fallen below 60 mm. and that neither returned to that point until expiration. The first of the hypotheses is excluded by the fact that no beating could be heard when the pressure in the cuff was raised above 60, and we have left only the assumption that there was an actual fall below 60 mm.

These relations are seen in the following table (table 1) which shows the results of three experiments performed on April 27, 1915.

TABLE 1

Effect of Valsalva experiments on systolic blood pressure; the fall

SUBJECT	P. M. D.	P. M. D.	P. C. H.
Systolic pressure	116		108
Pressure in cuff	90	60	90
Pulse disappears	1.6 seconds	14.8 seconds	10.8 seconds
Pulse reappears	7.3 seconds	29.9 seconds	33.0 seconds
Duration of effort			28.0 seconds

The time of disappearance and reappearance of the pulse is counted from the beginning of the expiratory effort.

Second method: The cuff of the Erlanger sphygmomanometer was adjusted and inflated until it stood (at various levels at different times) below the diastolic pressure. At each level the Valsalva maneuver was performed.

The following protocol is typical:

April, 1914; Dawson subject; Professors Eyster and Meek observers; blood pressures of subject while sitting on stool with knees drawn up and body bent forward 110 to 85 mm. At a given signal a long breath was taken followed by compression of the abdomen against the thighs and forced expiration with effort against the closed glottis. The lever ceased to show pulsations when the pressure in the cuff stood at 70 mm. A second trial was made with the pressure at 60 mm. This time pulsation became feeble and disappeared altogether when the straining was increased a little.

From this it is inferred that the systolic pressure had fallen below 60 mm. though how much below it was impossible to say. It should be remembered that the minimum movements of the lever do not

record systolic pressure but occur when the pressure in the cuff is considerably above systolic pressure. It would seem therefore that the systolic pressure was certainly somewhat below, perhaps considerably below 60 mm. It may be noted in passing that the heart sounds could not be heard with a stethoscope applied to the chest wall during the height of these experiments.

On the cessation of the effort the lever almost immediately began to move and the returning pulse wave soon showed the separation of the ana- and catacrotic limbs announcing that the systolic pressure had become equal to that in the arm cuff.

Secondary rise (1). It has been stated that after the fall of pressure described in the preceding section, a rise begins and before the end of the expiratory effort attains a considerable magnitude, as much even as 200 mm. Hg. The writers were unable to verify this assertion. It is true a rise above normal may occur, but we have not found it more extensive than that produced by simply holding the breath which seems to justify the assumption that our secondary rise is only a manifestation of asphyxia. In testing this matter the subject was required to perform the following maneuvers separately: *a*, the Valsalva experiment, the effort being either moderate or severe; *b*, holding the breath with the thorax in the expiratory position; and *c*, the same with chest in a position of very moderate expansion. The negative results are shown in the accompanying table (table 2).

Feeling reluctant to leave the matter of the secondary rise in this unsatisfactory condition, an effort was made to draw further light from animal experiments. The mode of procedure was to increase the intrathoracic pressure in dogs by sudden inflation of the lungs with compressed air. In a typical experiment this resulted in a rise followed by a fall of the mean blood pressure as indicated by the mercury manometer connected with the carotid artery. The rise was slight and brief (8 mm. in 5 sec. from the beginning of the inflation); the fall was extensive and prolonged (92 mm. in 73 sec.). Then because of Bruck's contention that the secondary rise is dependent upon an excess of abdominal over intrathoracic pressure, the abdomen was violently compressed by hand so that the mean blood pressure rose considerably (60 mm.); on relaxing the pressure on the abdomen the mean blood pressure fell, but not quite so low as before. After repeating the maneuver three times the dog became violently dyspneic and the mean blood pressure rose until it was nearly equal to the initial pressure (less by 15 mm.). The distention was then discontinued and the

pressure rose above normal but not much above normal (6 mm.). The accompanying kymogram (fig. 1) shows these relations in this particular experiment.

In a second experiment the blood pressure rose rapidly from normal (150) to 161 mm. Hg., then fell to 98 mm., and rose slowly until it reached 124 mm. Hg. Immediately after the pressure in the lungs was

TABLE 2

Effects of Valsalva experiment and of simple holding of the breath upon systolic pressure in three subjects

NUMBER OF EXPERIMENT	SUBJECT	SYSTOLIC BEFORE EXPERIMENT	SYSTOLIC PRESSURE DURING EXPERIMENT	DURATION OF EXPERIMENT	KIND OF MANEUVER
				seconds	
1	P. M. D.	109	118, 140	20	Valsalva severe
2	P. M. D.		120, 140, 140	40	Valsalva severe
3	P. M. D.	108	140	60	Valsalva moderate
4	P. M .D.		140	100	Breath held, expiratory
5	P. C. H.	110	130, 130	35, 40	Valsalva moderate
6	F. J. H.	100	126, 130	70	Valsalva severe
7	P. M. D.	112	125, 125, 125, 130	100	Valsalva severe
8	P. M. D.		130	25	Breath held, expiratory
9	F. J. H.		130	25	Breath held, inspiratory
10	F. J. H.		130	25	Breath held, inspiratory
11	F. J. H.	112	120	20	Breath held, expiratory
12	P. C. H.	109	130, 106, 118	65	Valsalva severe
13	P. C. H.		100, 96, 100	15, 40, 60	Valsalva severe

The figures 100, 96, 100 and 15, 40, 60 given for experiment 13 indicate successive determinations of systolic pressure and the times at which they were respecively made in a single Valsalva effort. The same method of presentation is followed in several of the other experiments in this table. Frequently only the total duration of the effort is noted although several determinations of systolic pressure were made, e.g., experiment 7. *Note that during the effort there is no rise in systolic pressure comparable to that described by Bruck, e.g., 160 to 200 mm.*

released, the blood pressure rose to 168 mm. Hg. From this point it gradually returned to normal.[4]

The writers are therefore still uncertain how to obtain the secondary rise described by Bruck, although a suggestion is offered below (p. 505).

[4] The writers wish to acknowledge the assistance of Dr. Clarence J. Brown in these animal experiments.

The pulse rate

During the height of an energetic Valsalva experiment, the radial pulse becomes imperceptible (Valsalva) and upon auscultation the heart sounds are found to be very indistinct or even inaudible (E. H. Weber). It is therefore often impossible to determine the heart rate by either palpation or auscultation and consequently the string-galvanometer was resorted to. The electrodes (large lamp wicks saturated with physiological salt solution) were bound about the forearms. A short control record was made and then at a word of command the subject began to strain and simultaneously a mark was placed upon the record. When the subject desired to cease from his effort, he did so with a loud vocal sound and the observer at once recorded upon the electrocardiogram. The electrocardiographic clock-work continued to run for a short time after the cessation of the effort. It was then stopped but after an interval of thirty seconds was started again for the purpose of making a final observation.

a b b b c d

Fig. 1. Effect of inflating lungs of dog with compressed air and of abdominal compression. The *uppermost line* shows the respiratory rate and amplitude as recorded by means of a drum-shaped pneumograph fastened about the body of the animal by an inelastic band. On inspiration the heads of the drum were pulled out. The pneumograph was connected with a recording tambour. Down strokes are inspirations. The extensive variations of this line which occur during the period of inflation of the lungs are merely mechanical, due to the manipulations of the experiments. The *second* line records the blood pressure, the cannula being in the carotid artery, the recording apparatus being the mercury manometer. The *third* line is the chronographic record (seconds). *a*, inflation begins; *b*, abdominal compression; *c*, respiratory efforts; *d*, inflation ends. *Note the effect of inflation (rise of pressure followed by fall), effect of compression of abdomen repeated three times (rise but not above normal), onset of dyspnea, cessation of inflation with a slow rise of pressure above normal.*

The electrocardiogram showed the following sequence of events: (1) a preliminary quickening;[5] (2) an initial slowing; (3) an accele-

[5] It should be remarked that the writers attribute no difference in meaning to these two terms (quickening and acceleration). The contrast is made merely for the sake of avoiding confusion.

ration; (4) a subsequent slowing which follows the cessation of the effort. Of these changes the subsequent slowing is the most constant; the initial slowing the least so. Thirteen experiments were performed on seven subjects.

1. Preliminary quickening. This fairly constant phenomenon is attributable to the deep inspiration which precedes the Valsalva experiment (cf. p. 482). It is the usual quickening which the heart normally exhibits as the accompaniment of inspiration. It differs only

TABLE 3

Changes in heart rate resulting from Valsalva experiment

SUBJECT	PRELIMINARY QUICKENING	INITIAL SLOWING	ACCELERATION	SUBSEQUENT SLOWING
Dawson no. 1	Absent	Absent	80	170
Dawson no. 2	Absent	Absent	50	Absent
Eyster	88	117	88	125
F. J. Hodges I	81	Absent	50	185
P. C. Hodges II	80	133	73	140
Kolls I	80	123	Absent	143
Kolls II	87	Absent	Absent	143
Kolls III	88	114	Absent	132 (1)
Menninger I	85	117	56	156
Menninger II	?	120	55	155
Menninger III	88	123	66	142
Nause I	80	116	64	133
Nause II	70	Absent	56	122
I Average	82.7	120	63.8	145
II Average	85	112	72	142

Thirteen experiments on seven subjects have been recorded. In the *first* column are shown the names of the subjects and the designations of the different experiments performed upon them; in the *second*, the percentage of the preliminary quickening, the normal heart cycle being regarded as 100; in the *third to fifth* columns, the initial slowing, acceleration, and subsequent slowing are similarly dealt with. For (1) see page 505. No change of less than 10 per cent is regarded as exceeding the limits of normal variation and consequently such are designated "absent" in the table. The interrogation mark indicates that the electrocardiogram was imperfect so that the particular point designated could not be determined. The first average is that of experiments in which a change took place; the second that of all thirteen observations, "absent" being reckoned as 100. *Note relative constancy of subsequent slowing and variability of preliminary quickening, of initial slowing and of acceleration.*

in being more pronounced just as the inspiration itself is more pronounced than usual. This quickening was obvious in ten experiments out of twelve (table 3). The maximum reduction in the length of the cardiac cycle was to 70 per cent of its normal value.

2. Initial slowing. In eight cases out of thirteen there occurred either simultaneously with the beginning of the effort, or very soon after its beginning, a cardiac slowing which was sometimes very marked, the maximum increase in the cardiac cycle being 33 per cent (figs. 2 and 3). The duration of the slowing was short in every case, its greatest length being ten beats. The increase in rate which followed was often abrupt, giving a sharp angle to the charted curve (fig. 3). Since

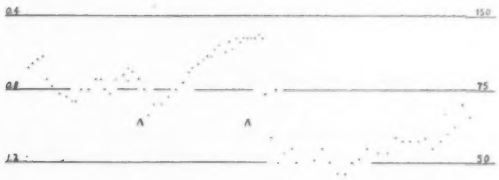

Fig. 2. The "typical" effect of the Valsalva experiment upon the heart rate. Each dot represents a single heart beat. Time is represented by distances along the abscissa; the numerals arranged in vertical column on the left represent the duration of the cardiac cycle in hundredths of a second; those in vertical column on the right represent the number of beats per minute corresponding to the figures in the first column. Of the wedges pointing upward that to the left indicates the beginning of effort, that to the right the end of the effort. Vertical broken line represents interval of 30 seconds between first and second part of figure. These explanations apply also to figures 3 to 5 and 8 to 15. Subject: Menninger (I). *Note preliminary quickening, initial slowing, acceleration and subsequent slowing* which has not passed off at the end of thirty-three seconds.

the period of slowing comes so soon after the beginning of the effort, one would *a priori* expect that it would coincide with the preliminary rise in systolic pressure. This expectation is borne out by the radial sphygmogram, for here the slowing of the pulse is often seen to accompany the reduction of the dicrotic elevation (*vide infra*). Even when the effort is so severe as ultimately to suppress the pulse at the wrist during the fall of pressure, still during the part of the effort in which this slowing occurs, the pulse continues to be palpable.

3. Acceleration. Ten of the thirteen subjects showed a period of cardiac acceleration which varied much in amount, the maximum reduction in the length of the cycle being to 50 per cent. This phe-

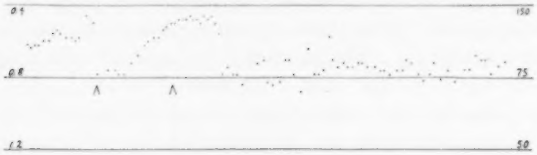

Fig. 3. "Typical" effect of Valsalva experiment upon heart rate. Subject: P. C. Hodges (II). For explanation of the symbols see legend of figure 2. *Note preliminary quickening, enormous initial slowing, relatively small acceleration and moderate subsequent slowing,* which has not disappeared at the end of forty-two seconds.

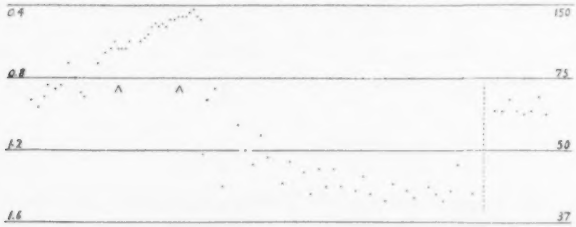

Fig. 4. Less "typical" effect of the Valsalva experiment upon the heart rate. Subject: F. J. Hodges (I). For explanation of the symbols see legend of figure 2. *Note preliminary quickening, absence of initial slowing, presence of acceleration and of very pronounced subsequent slowing,* which has returned to within nominal limits by the end of seventy-five seconds.

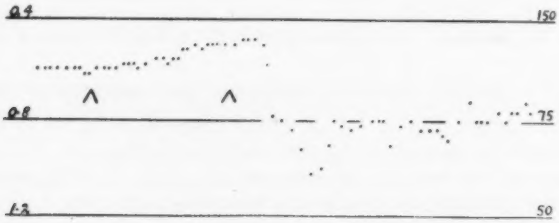

Fig. 5. Less "typical" effect of the Valsalva experiment upon the heart rate. Subject: Dawson (1). For explanation of the symbols, see legend of figure 2. *Note the absence of preliminary quickening and of initial slowing, presence of slight acceleration, and marked subsequent slowing,* which has not disappeared by the end of thirty seconds.

nomenon usually begins during the effort and continues from one to ten beats after the effort has ceased. It occurs, moreover, at about the time that the fall in blood pressures might be looked for, and might therefore be thought to be the accompaniment (or result) of the low pressure. Here again the sphygmogram confirms the a priori expectation in that the acceleration and the dicrotism are synchronous.

4. Subsequent slowing. In all but one experiment there occurred at from one to ten beats after the end of the effort, a very marked slowing of the pulse (figs. 2 to 5). Here the maximum lengthening of the cycle was to 185 per cent. The slowing was marked but usually not maximal at the outset. This final period was the longest of all, the slowing continuing often for forty or fifty beats and returning to normal sometimes with marked oscillations.

Character of the changes in rate

Duration of Q-T complex. If we assume that the duration of ventricular systole corresponds to the time interval between the Q- and T-waves of the electrocardiogram, then it is possible to determine the degree in which each of these phases of the cardiac cycle shares in the variations in duration of the complete cycle. A study of the electrocardiograms undertaken to elucidate this point shows that the variations are chiefly diastolic in character. The measurements in three typical experiments are presented in table 4.

Duration of the atrio-ventricular interval. Detailed study of the electrocardiogram was an after-thought. The string galvanometer was resorted to originally for the purpose of determining the heart rate after other methods had failed. The tracings were for the most part of little more value than was needed for the purpose of counting the heart beats, no efforts being made to cut out adventitious vibrations. In several experiments, however, the A-V interval could be made out.

This interval was occasionally altered. With a quickened heart beat there might be shortening of the A-V interval while with a slowing, the interval might be prolonged. Usually, however, the interval remained unchanged. The results relating to these points are embodied in the accompanying table (table 5). At one point (24 seconds) in the tracing obtained from F. J. Hodges (I) the P-wave could not be found. The heart was at this time beating very slowly and the picture was that of a nodal rhythm. The tracing is unfortunately not suffi-

ciently distinct to justify confidence, although suggestive enough to encourage further inquiry. In other tracings of hearts beating with equal slowness, the P-wave was clearly marked.

TABLE 4

Effect of Valsalva experiment on duration of Q — T complex

SUBJECT, ETC.	SECOND	DURATION OF		
		Cycle	Ventricle systolic	Ventricle diastolic
Dawson, no. 1, 6–20 seconds	49	1.00	0.24	0.76
	65	0.76	0.26	0.50
	5	0.60	0.28	0.32
	23	0.58	0.26	0.32
Dawson, no. 2, 6–19 seconds	23	0.50	0.23	0.27
	4	0.82	0.26	0.56
	29	0.82	0.26	0.56
	16	0.50	0.24	0.26
Menninger, II, 16–34 seconds	51	1.26	0.24	1.02
	50	1.20	0.24	0.96
	52	1.20	0.24	0.96
	12	0.80	0.24	0.56
	10	0.78	0.24	0.54
	11	0.74	0.24	0.50
	34	0.48	0.24	0.24
	34	0.48	0.24	0.24
	35	0.46	0.24	0.22

The *first* column shows the name of the subject to which is added a numeral indicating to which of the two or more experiments on this subject reference is made, also the numerical designation of the seconds at which the effort began and ended respectively, e.g., in Dawson no. 1., effort was begun during the 6th second and ended in the 20th after the beginning of the experiment. In the *second* column are the numerical designations of the seconds corresponding to the cycles analyzed in the succeeding columns. The *third, fourth and fifth* columns are the analyses of cardiac cycles of different lengths, taken from all parts of the electrocardiograms. *Note that duration of the cycle is mainly dependent on variations in diastole; while variations in systole are small and inconstant.*

Variations in the Q-, R- and S-waves of the electrocardiogram. The electrocardiograms were also examined to ascertain whether during the changes in rate there were any accompanying changes in the Q-R-S-complex. Nine tracings were found appropriate for this study (table 6). The changes observed were of three sorts, *a*, a general reduction

TABLE 5

Effect of Valsalva experiment on atrio-ventricular interval

SUBJECT, ETC.	SECONDS	CYCLE	A-V INTERVAL
Menninger I, 17–34 seconds, April 8, 1915	7	0.86	0.12
	8	0.86	0.12
	35	0.48	0.12
	48	1.20	0.12
P. C. Hodges I, 10–23 seconds	8	0.60	0.12
	31	0.84	0.12
Menninger III, 23–69 seconds	2	0.76	0.15
	28	0.96	0.15
	69	0.74	0.15
	78	1.14	0.15
Kolls II, 16–48 seconds, April 20, 1915	9	0.70	0.12
	63	0.80	0.12
Menninger II, 5–21 seconds, April 8, 1915	1	0.80	0.12
	21	0.48	0.12
	25	1.58	0.18
Kolls III, 14–43 seconds, April 20, 1915	6	0.72	0.13
	24	0.56	0.09
F. J. Hodges I, 12–20 seconds, April 10, 1915	6	0.72	0.12
	7	0.80	0.12
	8	0.88	0.12
	23	0.48	0.08
	24	1.22	0.00
	37	1.32	0.12

Here the *first* column gives the name of the subject, the date of the experiment and the period of effort. The last is indicated by two hyphenated numbers, the first being the second during which the effort was begun, the second that during which the effort was ended. The *second* column gives the serial number of the second corresponding to the values which follow it on the same line; the *third* and *fourth*, the duration in seconds of the cardiac cycle and atrio-ventricular interval respectively. For example, in Menninger I thirty-five seconds after the beginning of the experiment (that is, during the second which followed the cessation of the effort) the duration of the cardiac cycle was 0.48 second, while that of the atrio-ventricular interval was 0.12. *Note that occasionally atrio-ventricular interval varies in same direction as duration of cycle.*

TABLE 6

Changes in the Q-, R- and S-waves due to the Valsalva experiment

NAME, ETC.	PER CENT	R- AND S-WAVES		S-WAVE	Q-WAVE
		Preliminary quickening	Acceleration		
F. J. Hodges I, 13–20 seconds.....	50	10 0.72	20 0.48	21 23? 0.46 0.48	
Kolls I, 18–32 seconds.....	80	14 0.62	36 0.80		
Kolls II, 16–48 seconds..	87	14 16 0.56 0.60	26 43 0.60 0.68		
Menninger I, 17–34 seconds.....	56	14 0.68	34 0.48	21 34 36 0.68 0.48 0.48	
Menninger II, 4–21 seconds.....	55	3 7 0.78 0.86		10 21 23 0.80 0.46 0.48	
Dawson, no. 1, 6–19 seconds.....	80	5 7 0.62 0.60	20 21 0.48 0.48		7 22 0.60 0.58
Dawson no. 2 5–19 seconds.....	50	3 6 0.82 0.60	18 20 0.50 0.44		12 23 0.60 0.58
P. C. Hodges II.....	73				
Nause II.....	56				

The *first* column shows name of subject, number of experiment on that subject and duration of effort, i.e., serial number of seconds during which effort began and ended; the *second* gives maximum reduction of duration of cardiac cycle, i.e., percentage of normal cycle to which the duration fell. The *third* and *fourth* columns are best explained by these examples: In Hodges I a decrease in the R- and S-waves began at 10th second (during preliminary quickening) and stopped at 20th second (during acceleration). Below figures 10 and 20 are figures 0.72, 0.48 indicating duration in seconds of corresponding cardiac cycles. A second example is that of Kolls II. Here there were two separate periods of reduction of waves in question, one during the preliminary quickening and one during the acceleration. The *fifth* column shows beginning of increase of S-wave, viz., 21, 21, 10, and disappearance of increase, viz., 23?, 36, 23. Where there are three consecutive figures as 21, 34, 36, then 21 denotes beginning of increase, 34 a sudden rise in the degree of increase and 36 disappearance of increase. Durations of corresponding cycles are placed below these figures. The *sixth* shows beginning and end of increase of Q-wave. *Note more constant presence of reduction of R-S waves, and relative infrequency of increase of Q-wave.*

in the size of the R- and S-waves; *b*, an increase in the Q-wave; *c*, an increase in the S-wave sometimes accompanied by a simultaneous decrease in the R-wave. No changes were observed when the heart became slower than normal but only when it quickened its pace. The general reduction in size occurred in seven experiments. In three of these the decrease began with the preliminary quickening and con-

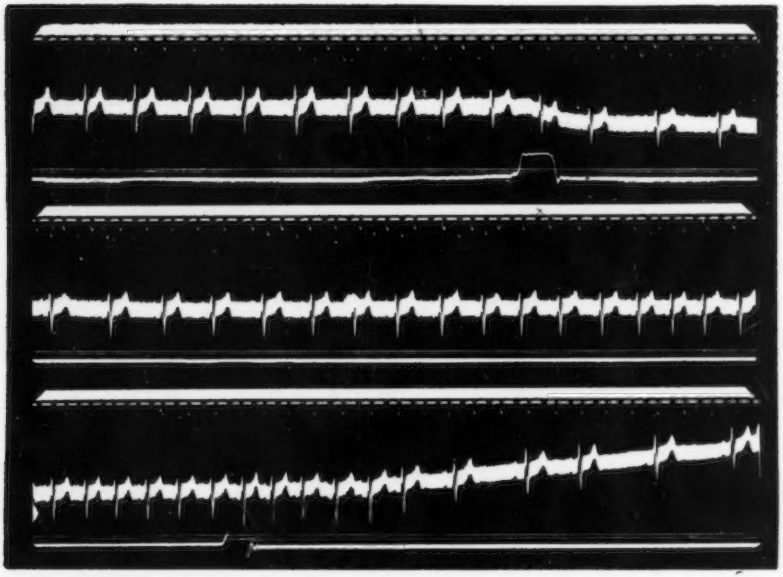

Fig. 6. Electrocardiogram taken during a Valsalva experiment. Subject: Menninger (II). Upper line is a chronographic record showing fifths of a second; *middle* line is the electrocardiogram; *lower* line, signal showing beginning and end of effort. *Note preliminary quickening, initial slowing, acceleration with increase in S-wave, and subsequent slowing.*

tinued all through the effort and for a few seconds after the effort was over. In three other experiments the decrease accompanied the preliminary quickening, then disappeared again during the acceleration. This change seems therefore not to be the result of the Valsalva experiment as such, but merely the effect of decreased vagal tonus. Finally in one case the change appeared during the preliminary quickening only.

The other two changes both imply an increase of the negativity of the apex as compared with the base of the ventricle. The increase in the Q-wave occurred in two experiments on the same subject. In one of them (Dawson no. 2) the phenomenon was very marked; in the other (Dawson no. 1) it was distinctly observable although less pronounced. The change began during the effort and persisted for a few seconds after the cessation of the latter. It should be noted that in neither of these experiments was there a preliminary quickening.

The increase in the S-wave sometimes began at the time of cessation of the effort (one experiment) and always lasted until the acceleration gave place to the subsequent slowing. It might, however, begin much earlier in the effort (two cases). It never accompanied the preliminary quickening and may therefore be inferred to result from the mechanical conditions of the Valsalva experiment itself.

In the present unsatisfactory state of our knowledge regarding the cause of the Q-, R- and S-waves and the significance of their variations, the writer feels that all he is justified in doing is to record the foregoing facts without attempting an interpretation. It is true that the tables might be turned and the facts in question might be utilized for the elucidation of the reverse problem, namely, the meaning of the Q-, R- and S-waves, but for dealing adequately with the latter further experimentation along these lines is desirable.

The sphygmogram

Owing to some curious statements in the literature in regard to the significance of the sphygmogram obtainable during Valsalva's experiment (4), it seemed well to devote some attention to this matter. Of several sphygmograms obtained one is given as an example (fig. 7). Here it is seen that the normal pulse waves undergo a series of changes which may be described as follows: *a*, a fall of the base line; *b*, elevation of the base line, decrease in size of the beats and obscuration of dicrotic wave; *c*, fall of base line but not to normal, hyperdicrotism, and continued small size of pulse wave; *d*, return to normal by decrease in dicrotic wave, fall in base line and increase in size of pulse. One finds no difficulty in interpreting these changes: *a*, The fall in the base line is the result of the strong inspiration with which the Valsalva effort begins and which assists in emptying the radial venae comites. *b*, The rise in the base line is due to distention of the radial artery resulting from the rapid rise in arterial pressure and also to the filling of the

venae comites resulting from the rise in intrathoracic pressure which obstructs the venous outflow from the arm. *c*, As the arterial pressure falls rapidly, the base line sinks but does not reach normal since the veins are still engorged. *d*, With the return of normal pneumatic conditions within the thorax, the veins empty rapidly and the base line falls to normal. The hypodicrotism and the hyperdicrotism are evidences of high and low blood pressure respectively.

These explanations, as far as they go, seem to the writers to accord well with known facts and to render the efforts of some investigators not a little superfluous.

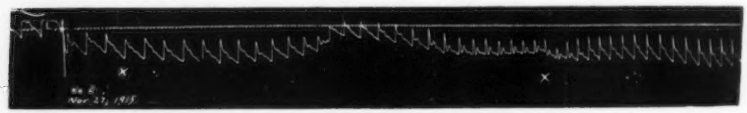

Fig. 7. Radial sphygmogram during Valsalva experiment. Chronograph records fifths of a second. *x* indicates approximately beginning and end of experiment including preliminary slow and deep inspiration. *Note variations in base line (an index of engorgement of radial vessels), and in height of dicrotic elevation (an index of arterial pressure).*

LIFTING

It has been the view usually accepted that lifting and the Valsalva experiment are essentially alike insofar as the disturbances of the circulation are concerned. This statement is in the main correct. The blood pressures and pulse rate usually do show a picture which corresponds to that seen in the Valsalva experiment although often these changes are less pronounced. As in the case of the Valsalva experiment, there are two series of variations to be considered, the blood pressure changes and the variations in the pulse rate. We shall consider these in turn.

Blood pressure (systolic)

The blood pressure changes will be rather summarily dismissed, otherwise, because of the essential resemblance between the effect of lifting and that of the Valsalva maneuver, we would only be engaging in vain repetitions. Three experiments were performed and showed the *primary rise* and the *fall* but no second rise until after the cessation of the effort.

It is, however, highly desirable that these studies should be carried further. Enough material has not been gathered to enable the writer to study individual variations.

Pulse rate

The pulse rate on the other hand received adequate attention. Its behavior also resembles in the main that observed in the Valsalva experiment. There are, however, a few exceptions as will be seen presently.

The method employed was as follows: The subject stood upon a strong square piece of wood (about 80 x 80 cm.) to the center of which was fastened by a hook a registering spring dynamometer. The subject stood over the hook and passed around the body and over one shoulder a sling which was secured to the free pole of the dynamometer. The sling was then shortened until the subject stood with the knees slightly bent. At the word of command the legs were extended and the subject made a vigorous attempt to lift the square piece of wood upon which he stood. The amount of effort was registered by the dynamometer. The subject ceased his effort with a loud gasp which was recorded by the operator who had also marked upon the electrocardiographic tracing the moment at which the command to lift had been given. During the whole of this procedure electrocardiograms were being taken from the two forearms.

The alterations of the pulse rate are presented best by means of the accompanying table (no. 7) and charts (8 to 15). The former contains most of the data embodied in the discussion while the latter are shown chiefly for the purpose of illustration.

On perusing table 7 one becomes aware of the following facts: *a*, In general the changes in heart rate are similar to those produced by the Valsalva experiment, but are quantitatively less. *b*, The preliminary quickening is a very inconstant phenomenon. This accords with the supposition already advanced that the preliminary quickening is due to an accentuation of inspiration for it often occurs that one does not precede an effort at lifting by taking a deep breath. *c*, The initial slowing occurred in numbers 1 and 4, but was absent from numbers 6 and 12. This may indicate that the phenomenon in question is at least partly dependent upon the severity of the lift for the dynamometer record was lower in numbers 6 and 12 than in numbers 1 and 4. *d*, The subsequent slowing is one of the most constant features; its

appearance may however be delayed for as much as one minute (nos. 5 and 10). It is not always the last sign to disappear when the effort is reduced for although this would seem to be the case sometimes (no. 12) it is not always so (no. 15). *e*, The acceleration is also a pretty constant feature. It may extend throughout most of the period of effort (nos. 5 and 7). On increasing the effort, it may linger after the secondary slowing has disappeared or on decreasing disappear before it (nos. 15, 12). *f*, The erratic records (nos. 7, 13 and 14) require special con-

TABLE 7

Changes in heart rate due to lifting

NUMBER	SUBJECT, ETC.	PRELIMINARY QUICKENING	INITIAL SLOWING	ACCELERATION	SUBSEQUENT SLOWING
1	Hodges I	?	126	Absent	166
2	Jolivette	89	Absent	Absent	139
3	H'Doubler II	88	Absent	82	123
4	Pressentin I*	Absent	120	89	120
5	Bodman	Absent	Absent	80	116 (1)
6	Hodges II	85	Absent	66	134
7	Dawson I	Absent	Absent	86 (2)	140
8	Schlatter*	Absent	Absent	75	159
9	Kriskey	Absent	Absent	70	Absent
10	Dawson II*	Absent	Absent	79	152 (3)
11	Morris	87	Absent	78	177
12	Pressentin II	Absent	Absent	Absent	113
13	H'Doubler I	?	Absent	97, 114 (4)	125
14	Kelley*	?	Absent	121, 146 (5)	128
15	Dawson 18	Absent	Absent	74	Absent
16	Dawson 19	Absent	Absent	60	114
I Average		87	123	76	136
II Average		96	102	81	131

The *first* column gives reference numbers which are used in discussion in text For remaining columns see legend of table 3. No change of less than 10 per cent is regarded as exceeding the limits of normal variation and consequently such changes are designated "absent" in table. Interrogation point and averages as in table 3. (1) Signifies maximum change reached late (60 seconds); (2) interrupted by a slowing (see page 505); (3) coming on late; (4) omitted from both averages; here a slight acceleration occurred (97 per cent) but the last and longest part of effort was accompanied by slowing (114 per cent maximum); (5) omitted from both averages; here usual acceleration was replaced by slowing, shortest cycle during effort was 121 per cent, the longest 146 per cent. In numbers 1, 4 and 15 effort was much more severe than in numbers 6, 12 and 16 respectively. *Shown in figures. *Note that changes although essentially similar to those due to Valsalva experiment are less in amount.*

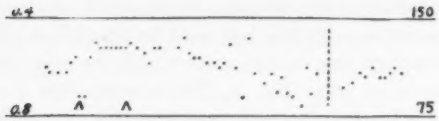

Figure 8. More "typical" effect upon the heart rate of lifting. Subject: Pressentin (I). For explanation of symbols see legend of figure 2. *Note the initial slowing, acceleration and subsequent slowing.*

Fig. 9. Less "typical" effect of lifting upon the heart rate. Subject: Schlatter. For explanation of the symbols see legend of figure 2. *Note the absence of the preliminary quickening, and the initial slowing. The acceleration and subsequent slowing are still pronounced.*

Fig. 10. Less "typical" effect of lifting upon the heart rate. Subject: Dawson (I). For explanation of the symbols see legend of figure 2. *Note the absence of the preliminary quickening and the initial slowing; the presence of two periods of acceleration separated by a slight slowing, and of the subsequent slowing.*

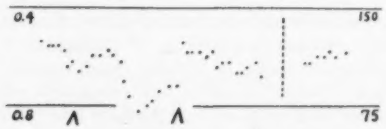

Fig. 11. "Atypical" effect of lifting upon the heart rate. Subject: Kelley. For explanation of symbols see legend of figure 2. It is exceptionally unfortunate that in this case the gap in the record (the half-minute pause) occurs where it does. It is possible that during this interval the usual secondary slowing may have taken place. *Note the presence of the initial slowing, of a return to normal following the cessation of the lift.*

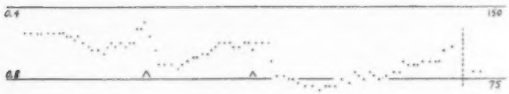

Fig. 12. First of three figures showing the effect upon the heart rate of prolonging the Valsalva experiment. Subject: Kolls (I). For explanation of the symbols see legend of figure 2. This short effort is presented for the sake of comparison with figures 13 and 14. All three were obtained from the same subject. *Note typical picture consisting of preliminary quickening, primary slowing, acceleration and subsequent slowing.*

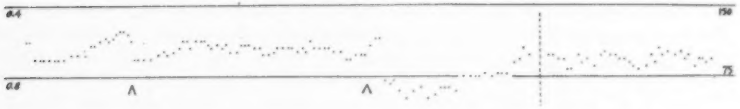

Fig. 13. Second of three figures showing the effect upon the heart rate of prolonging the Valsalva experiment. Subject: Kolls (II). For explanation of the symbols see legend of figure 2. This effort is longer (32 seconds) than that of the preceding figures (14 seconds). *Note preliminary acceleration, very slight primary slowing (which amounts merely to a return to normal), moderate secondary acceleration, subsequent slowing.*

Fig. 14. Third of three figures showing the effect upon the heart rate of prolonging the Valsalva experiment. Subject: Kolls (III). For explanation of the symbols see legend of figure 2. This long effort lasted sixty seconds. *Note the preliminary acceleration, the primary slowing, the acceleration, the subsequent slowing. There is also a period of slowing before the official end of the effort.*

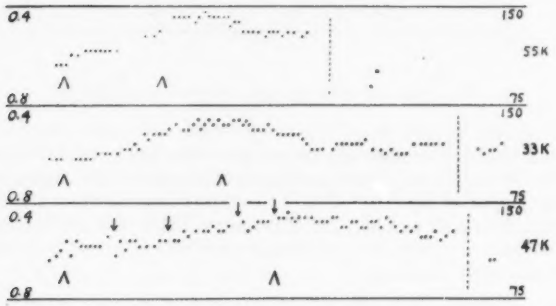

Fig. 15. The effect of lifting upon the heart rate as modified by the severity of the effort and by gasping. Subject: Dawson. For explanation of the symbols see legend of figure 2. The three curves are constructed on the same scale. The arrows pointing downward toward the third curve indicate the moments at which the subject gasped while making this record. The weights lifted which correspond to the three curves are, when arranged in order from above downward, 55, 35 and 47 kilos. *Note that the last curve shows no greater deviation from the normal than the second curve excepting the fact that the acceleration took longer to pass away.* This is *in spite of the greater effort* as compared with the second.

TABLE 8

Effect of lifting on duration of Q-T complex

SUBJECT, ETC.	SECOND	DURATION OF		
		Cycle	Ventricular systolic	Ventricular diastolic
Dawson II, 15–36 seconds	48	1.04	0.22	0.46
	39	0.80	0.22	0.24
	13	0.68	0.22	0.46
	26	0.48	0.24	0.24
Pressentin I, 3–10 seconds	22	0.68	0.24	0.44
	1	0.60	0.24	0.36
	12	0.52	0.22	0.30
Pressentin II, 3–30 seconds	36	0.64	0.24	0.40
	2	0.60	0.22	0.38
	27	0.56	0.22	0.34

For explanation see legend of table 4. *Note similarity of this table to corresponding table for Valsalva experiment (no. 4).*

sideration: *1*, The record no. 7 is easily interpreted by supposing that a fluctuation of the attention caused this subject to relax his efforts and then to reinforce them just before the cessation of the pulling. *2*, The record no. 13 might be explained in the same way, but for the fact that we would then expect the slowing to continue after the cessation of the effort until the final return to normal. If this objection be valid we are left without an explanation of this record. *3*, The record no. 14 is characterized by a great slowing during the effort. Here again one might urge that the subject ceased her efforts before she was aware of it, and that the actual termination of the effort does not coincide with the intentional termination. But the same objection that has just been advanced in the case of no. 13 applies here with redoubled force for the unusual slowing does not persist after the effort has come to its intentional conclusion but is actually replaced by a marked acceleration. The justifiable supposition is that the intentional termination of the effort is the actual termination of the effort, but if so the unusual slowing remains without an explanation (cf. pp. 505 and 485).

Duration of the Q-T complex. Three electrocardiograms were found suitable for this study and the results were similar to those occurring in the case of the Valsalva experiment (table 8).

Character of the changes in rate

Duration of the atrio-ventricular interval. What has already been said in regard to the unsatisfactory character of the electrocardiograms in the case of the Valsalva experiment applies with still greater force in the case of the lifting experiments. We shall however consider such results as have been obtained. Of seventeen experiments only one showed a P-wave of adequate distinctness, the rest being spoiled by vibrations due to adventitious alternating currents. This single record (Dawson I) showed no change in the atrio-ventricular interval although the cycle was shortened from 0.64 to 0.44 seconds. These cycles occurred in the second and seventeenth seconds respectively, the period of effort being from the third to the twelfth seconds.

Variations in the Q-, R- and S-waves of the electrocardiogram. As in the case of the Valsalva experiments the electrocardiograms were also examined to ascertain whether during the changes in rate there occurred any modifications of the Q-, R- and S-waves. Ten of the seventeen experiments were found satisfactory for this study and the three changes observed in the case of the Valsalva experiment were noticed, and shall now be considered in order.

1. General decrease in the size of the R- and S-waves. Five of the experiments were negative while four gave results similar to those obtained in the Valsalva experiments (table 6). The remaining experiment (Kelley) differed entirely from the rest. Here the maximum acceleration was to 94 per cent but the record showed that during

TABLE 9

Changes in the R- and S-waves due to lifting

NAME, ETC.	PER CENT	R- AND S-WAVES	
		Preliminary quickening	Acceleration
Kelley, 4–14 seconds	94 (144)	(3) (0.36)	(14) (0.68)
Jolivette	92	5 7 0.56 0.54	10 16 0.60 0.66
Pressentin II	90	No change	No change
H. Doubler II	84	3 0.60	22 0.60
Pressentin I	80	No change	No change
Bodman	79	No change	No change
Dawson 18	74	No change	No change
Kriskey	70	6 0.56	19 0.46
Dawson II, 15–36 seconds	67	15 0.64	36 0.60
Dawson 19	65	No change	No change

For explanation see table 6. The figures in parentheses are the discordant results (Kelley) referred to in text. *Note similarity of this table to corresponding table for Valsalva experiment (no. 6).*

the greater part of the lift the duration of the cycles was increased, attaining a maximum of 144 per cent. Along with this increase in duration of cycle there was a marked increase in the R-Q complex lasting from the third to the fourteenth second. No explanation is offered at the present time for this phenomenon.

2. Increase in the S-wave was not found at all, and

3. Increase in the Q-wave was found in only one instance at the period of maximum acceleration when the duration of the cycle had been reduced from 0.58 to 0.46 seconds.

CERTAIN MODIFICATIONS OF LIFTING AND THE VALSALVA EXPERIMENT

The effect upon the heart rate of prolonging the Valsalva experiment was studied in a single case. (Figs. 11, 12 and 13.) Here the usual four phases appeared in the first, the shortest, effort. On lengthening the period of effort there was no conspicuous change in the character of the results. But when the period was still further lengthened there appeared a marked slowing before the recorded end of the effort. On this record is marked the only *intentional* termination of the effort but if the attention or strength of the subject should flag during the experiment and the effort therefore be relaxed, a considerable amelioration of the pressure conditions within the thorax would result without the subject becoming aware of it. This state of things might give evidence of its existence through a premature slowing. The latter would disappear again when the effort was reinforced as it would be prior to the intentional abandonment of the effort which brings the experiment to its official termination.

Support is given to this explanation by the somewhat similar results shown in figure 10. Here the writer (Dawson) was the subject and was watchful of his mental states during the course of the experiment (lifting). A fluctuation in the degree of effort was synchronous with a premature slowing which was succeeded by an acceleration followed in turn by a second slowing, the one which we have been designating the subsequent slowing.

It has been seen that the pulse variations are of two sorts, the preliminary slowing which coincides with a respiratory movement, and the other three phases which are synchronous with changes in pressure. The initial slowing accompanies the primary rise in pressure; the acceleration, the fall in pressure and the subsequent slowing, and the return of the blood pressure to or above normal. It is a suggestive thought although perhaps not a justifiable assumption at the present time to regard the changes in heart rate as indices of blood pressures. In such a thought or assumption it is implied that in Kolls III there was a *secondary rise* in systolic pressure comparable to that described by Bruck (1).

The effect upon the systolic blood pressure and pulse rate of gasping during the Valsalva experiment and during lifting was studied in several instances. This seemed desirable because of the fact that certain forms of physical effort are characterized by periods of strain interrupted by gasps. All very prolonged strains are so interrupted. Observations upon the blood pressure changes were confined to the Valsalva experiments while those dealing with the heart rate were concerned with lifting only.

In the former study the method employed was the following: The pressure in the cuff of Erlanger's sphygymomanometer was raised to a high level and allowed to fall slowly during the performance of a Valsalva strain interrupted by gasps. At every 5 mm. of fall the observer marked with a key upon the drum. When the record was completed the pulse curves were examined to locate the first appearance of pulse tracings showing one of the systolic characters, namely, the separation of the catacrotic from the anacrotic limb. The pressure (shown by the manometer) which was synchronous with this form of pulse wave was taken to be the systolic pressure at that moment.

In three experiments in which the manometer pressure fell from 180 mm. the systolic pressure was found to be 140, 130 and 130 mm. respectively, during gasps. The normal systolic pressure of the subject observed was 118 mm.

In studying the heart rate the usual electrocardiographic method was employed. The results obtained are shown in fig. 15. Three experiments were performed upon the same subject (Dawson). In the first 55 kilos were lifted, in the second 35, and in the third 47. The duration of the lift was progressively longer in the order given (10, 16 and 21 seconds respectively) and during the third effort the subject gasped repeatedly. As shown in the figure the changes in rate were greatest in the first experiment while those in the other two were about equal, the somewhat greater acceleration in the second being balanced by the greater subsequent slowing in the third. Thus the changes produced in the third experiment (with gasping) were no greater than those in the second in spite of the fact that in the third experiment the duration of the lift was 34 per cent longer and the weight lifted 37 per cent heavier.

LABOR PAINS

As already stated in the introduction, parturition resembles considerably the Valsalva experiment in its mechanics. There is here the same closure of the glottis and forced expiratory effort, and we may infer that the cardio-vascular reaction is similar in the two phenomena.

A chance observation upon a rabbit which was unexpectedly found to be pregnant during the course of an experiment seems worthy of being recorded. In one of a series of experiments on the blocking of nerve impulses through the application of heat, the sciatic of this rabbit which was under the influence of chloral, urethane and ether was stimulated with a moderate tetanic current. This stimulus was

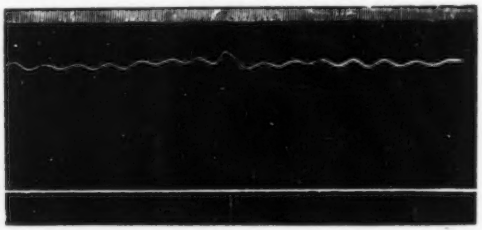

Fig. 16. Changes in respiration and mean blood pressure during labor. Subject: rabbit under chloral, urethane and ether. *Upper* line records respirations by means of tambour connected with trachea cannula; *middle* line, mean blood pressure recorded by means of cannula in carotid artery; *lower* line, seconds and zero pressure. Mean pressure before spasm 114 to 122 mm., at height of rise 128 mm., at depth of fall 110 mm. Hg. The record shows no change in rate of heart rate nor of respiratory movements. *Note the decrease and cessation of respiration, a rise followed by a fall in mean pressure.*

sufficient to start a series of labor pains which resulted in the abortion of several nearly-mature fetuses. As the carotid of the rabbit was at the time connected with a mercury manometer, a record of the mean blood pressure was obtained during these labor pains. The respiration was also being recorded by means of a side tube from the trachea cannula.

On examining this record (fig. 16) one observes a uniform movement of the respiratory recorder and of the mean blood pressure. The latter shows waves of the first (cardiac) and of the third order (vasomotor) (5). The waves of the second order (respiratory) are absent. From the normal level the mean pressure rises slowly for the

duration of about three of the large waves. It now rises sharply, next falls more slowly to a level which is subnormal and from which it returns to normal in the duration of about three of the large waves (sixty seconds).

These pressure phenomena are not dissimilar to those obtained in the case of the Valsalva experiment but since the closure of the glottis has been rendered ineffective by tracheotomy, the changes in question must result from a totally different set of causes. It is suggested that the cause in this case is the increase in peripheral resistance due to the contraction of the uterus. The writers have frequently observed a well marked and similar pressure change in the rabbit on stimulation of the peripheral end of the sciatic where the only area of constriction was the lower leg and foot and it seems not unnatural to suppose that these changes in pressure may readily result from the constriction and relaxation of so large and vascular an organ as the uterus at term.

During the sharp rise in mean pressure the respirations show a short increase, a diminution for several movements, a cessation for a couple of seconds and as the pressure falls a rapid return. The subsequent movements are at first supernormal but normality is reached in about thirty-five seconds. The respiratory pause seems to have been in inspiration for the last weak movement was inspiratory (a down stroke) and the first of the returning movements expiratory (an upstroke)

Under the conditions which existed in this animal, one would have expected the respirations to show first a deep inspiration followed at once by the strong although mechanically ineffective expiration. The phenomena observed, namely, gradual suppression and gradual return of the respiratory movements, are puzzling and the writers do not hazard a conjecture as to their significance. Suffice it then to emphasize that in constructing a picture of the blood pressure changes in parturition one must include changes which are independent of the closure of the glottis.

SUMMARY OF RESULTS

The following summary is submitted without further discussion. Credit to previous investigators together with the comment and criticism of one of us (D) will be reserved for the historical résumé already referred to (footnote 3, p. 482).

I. In the Valsalva experiment the following phenomena were observed:

1. The systolic pressure rose rapidly, primary rise (McCurdy). This was followed by an extensive fall (Valsalva and Weber).

2. In place of the extensive secondary rise described by Bruck (to 180–200 mm.) a rise to not more than 140 mm. was observed, i.e., a height readily accounted for by the mild degree of asphyxia which occurred in these experiments.

3. When in the anesthetized dog the inflation of the lungs had reduced the mean blood pressure by 92 mm., neither violent abdominal compression nor severe dyspnea raised this pressure above normal again. On relieving the inflation the mean pressure rose somewhat above normal.

4. On cessation of the Valsalva effort there was a moderate rise of the systolic pressure.

5. The pulse rate showed a preliminary quickening which accompanied the inspiratory movement (cardiac cycle was sometimes reduced by 30 per cent), an initial slowing (cardiac cycle was sometimes increased by 33 per cent), an acceleration (cardiac cycle sometimes decreased by 50 per cent). The last usually began during the effort and continued for several beats after the effort had ceased, often not reaching its maximum until that time.

6. Subsequent to the effort there was a slowing (cardiac cycle sometimes lengthened by 85 per cent) which might continue for 40 to 50 beats.

7. As the time which elapsed between the Q- and T-waves (electrocardiographic) showed but little variation, the changes in the cardiac cycle may be regarded as chiefly diastolic.

8. The atrio-ventricular interval was usually unchanged but might be shortened with the shortening of the cardiac cycle.

9. The R- and S- waves might be reduced during the preliminary quickening or the acceleration or both. The acceleration might be accompanied by an accentuation of the Q-wave, or by an increase of the S- with a simultaneous decrease of the R-wave.

10. The changes in the sphygmogram were readily attributable to the changes in venous and arterial pressure.

II. Lifting was characterized by the following phenomena:

11. The changes in systolic blood pressure were essentially similar to those which occurred in the Valsalva experiment.

12. The changes in pulse rate were often essentially similar to those occurring in the Valsalva experiment. They were, however, less regularly present and might be less in amount. In order of the decreas-

ing frequency of their occurrence they were *a*, subsequent slowing and acceleration; *b*, initial slowing; *c*, preliminary quickening.

13. The shortening of the cardiac cycle was chiefly diastolic. The R- and S-waves might show a simultaneous decrease. The Q-wave was sometimes accentuated. No increase in the S-wave was noted (with the exception mentioned on page 504, viz., no. 14).

III. When the Valsalva or lift was modified, the usual picture was somewhat changed as follows:

14. The effect upon the heart rate of greatly prolonging the Valsalva experiment was to decrease the intensity of the effort and with this the extent of the changes in the heart rate.

15. When the Valsalva experiment experienced a series of *interruptions* consisting of a single gasp each, the systolic blood pressure rose with each gasp 10 to 20 mm. above normal.

16. When a lifting experiment experienced similar interruptions, the changes of heart rate were less in extent than would otherwise occur even when the weight lifted was greater in amount.

IV. Observations made during parturition:

17. During a labor pain in the anesthetized and tracheotomized rabbit, the mean blood pressure experienced changes (rise followed by fall) which are attributable to the changes in peripheral resistance due to uterine contraction and subsequent relaxation.

18. The respiration also changed decreasing in amplitude to a standstill in the inspiratory phase and then gradually returning to normal.

The writers have much pleasure in acknowledging the assistance of those students of medicine and of physical education who served as subjects in this research. To the names already mentioned in the text should be added those of Misses Glassow and McFadden.

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SOME ASPECTS OF THE NEUROMUSCULAR RESPIRATORY MECHANISM IN CHELONIANS

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In 1795 Robert Towson, at Göttingen, first showed that tortoises do not swallow air, as had previously been believed, but that by the contraction of certain muscles, the lung is compressed and expels air, "then, ceasing to contract, the other muscle contracts and draws the former, (e.g., the lungs), within; thus a vacuum is formed into which the air rushes, as in the respiration of animals with a thorax" (1).

In 1863 Mitchell and Morehouse (2) described the normal respiration of the turtle as being due to the alternate contractions of certain antagonistic groups of muscles. "Inspiration is effected by the contraction of the flank muscles, which in appearance strongly resemble the diaphragms of superior animals. Expiration is effected by muscles which lie within the breast-box and consist of anterior and posterior bellies connected by a strong tendon continuous across the mid-line and common to both sides of the animal. These muscles act together and compress the viscera against the lungs." According to these authors the lung does not take an active part in the respiratory movements, but is compressed by the contraction of the expiratory muscles, while its passive expansion, due to the contraction of another set of muscles, causes more air to rush in and fill the lung cavity as in mammalian respiration. The same authors found that the neural apparatus of the respiratory mechanism consists essentially of the vagus supplying the larynx and lungs, of the spinal nerves distributed to the respiratory muscles of the trunk, and of the medulla oblongata through which the synchronous movements of the glottis and flanks are controlled. The functional activity of the vagus in connection with the lungs is not emphasized, however, in view of the passive rôle played by the lungs in normal respiration.

In 1878 Paul Bert made further observations, confirming the fact that respiration is carried on by contraction and dilatation of the

thoracic-abdominal cage. He also made the important observation that the lungs themselves can be made to contract by direct electrical stimulation (3).

François-Frank, in 1906, made a comprehensive and detailed study of the muscular mechanism of respiration in the turtle, with an analysis of the normal respiratory curves. He studied the lungs and their innervation by the vagus. He also observed spontaneous contractions of the lung tissue so long as the medulla was intact (4).

In 1908 Prévost and Saloz (5) called attention to the fact that stimulation of the vagus produces constriction of the bronchioles in the turtle, and in 1918 Jackson and Pelz demonstrated that while stimulation of the vagi produces constriction of the lungs, stimulation of the sympathetic chain with a very weak tetanizing current produces dilatation (6).

In this paper the experimental work under discussion is twofold, dealing with the activity of the lungs themselves under certain conditions, and the effects of experimental lesions of the cerebrum, optic lobes and medulla upon the respiratory muscles which are effective in maintaining normal respiration.

The activity of the lungs is first considered. Turtles were lightly etherized and the cranial cavity was opened. The cerebral hemispheres were removed, and the technique followed was that devised by Jackson in which, after removing the plastron, the fore and hind limbs were removed as well as all the viscera except the lungs and heart. The latter was connected with a recording lever. The vagi were freed for some distance in the neck, and ligatures were placed loosely around them. A small glass cannula was inserted in the trachea, by means of which, after being half inflated, the lungs were connected with a very sensitive recording tambour, which indicated very small changes in volume. A small pair of electrodes, insulated except at the very point, was then fastened firmly, by means of adhesive tape, into the cranial cavity on the optic lobes, which were then stimulated with a tetanizing current. In order to check up results as being due to conduction by nerve fibers and not to escape of current in the cranial cavity, whenever practicable before the close of an experiment the optic lobes were divided from the brain stem and again stimulated with an induction current of the same intensity as before.

Following is the protocol of a typical experiment.

May 20, 1919

Turtle prepared according to Jackson's technique.

Optic lobes stimulated for 20 seconds with medium induction current. Good contraction of the lungs. Repeated.

Right vagus stimulated with medium induction current for 20 seconds. Contraction of the lungs with inhibition of the heart. Repeated.

Right vagus divided.

Left bronchus occluded with artery clip, optic lobes stimulated. No contraction of right lung.

Right bronchus occluded, left released. Optic lobes stimulated. Contraction of left lung. Repeated.

Optic lobes divided from brain stem, then stimulated. No contraction of left lung.

With a moderate tetanizing current, upon stimulation of the optic lobes or the medulla, I have uniformly obtained a rise in the point of the recording lever, similar to that obtained by stimulation of the peripheral end of the vagus, except that there is no such inhibition of the heart as occurs in the latter case. As figure 1 shows, the heart beat is uninterrupted, although the contraction of the lungs is identical with that which accompanies inhibition of the heart on stimulation of the vagus. This contraction is long and slow with a long latent period—a very different type of response from a normal respiration, in which there is a sudden sharp contraction of the muscles of the thoracic-abdominal cage with an expulsion of about two or three times the volume of air expired in a simple contraction of the lungs. Inhibition of the heart occurs only when a current of greater intensity than that necessary to produce contraction of the lungs is employed. Neither stimulation of the optic lobes nor of the medulla oblongata has ever produced dilatation of the lungs.

The single contraction upon stimulation of the optic lobes or medulla with the tetanizing current is a typical smooth muscle contraction, such as one obtains from the urinary bladder of the cat when the hypogastric nerves are stimulated. Such a contraction as the result of stimulation may be shown with one lung by placing a light artery clip upon the main bronchus of the other side and thus occluding the opposite lung. If this is done, and the optic lobes are stimulated, a normal response is elicited from the side under observation. But if the vagus to this lung be now cut, and stimulation again be done, there is no contraction. If the bronchus on the side on which the vagus has been cut is now occluded with the artery clip and the other side is freed, on stimulation of the optic lobes there is a normal response from the normal side.

Such behavior apparently indicates that in the turtle the fibers of the vagus which pass to the lungs have anatomically different nuclei of origin from the fibers which go to the heart.

During a number of experiments, I observed in detail a phenomenon of which François-Frank speaks. This is the spontaneous rhythmic

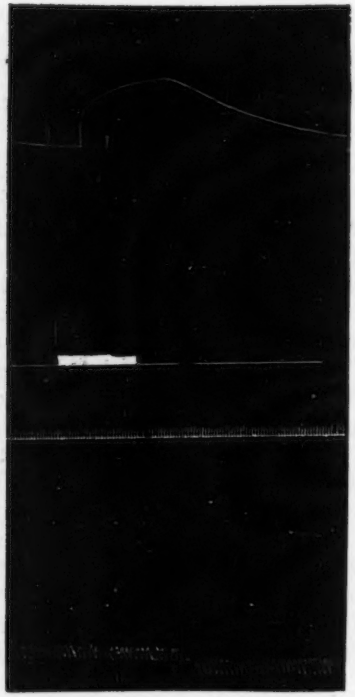

Fig. 1. Upper tracing, response of right lung (vagus intact) to stimulation of the optic lobes. Lower tracing, heart beat.

contraction of the lungs of the turtle similar to that evoked by stimulation of the brain stem. Unlike the spontaneous contractions of smooth muscle, which take place independently of a centrifugal mechanism, the medulla and vagi of the turtle must be intact,—a demonstration of the fact that a central origin and efferent fibers are necessary to produce the contractions.

We now pass to a consideration of the neuromuscular mechanism which is effective in maintaining normal respiration. In the turtle the muscular mechanism, as earlier authors have shown, is the thoracic-abdominal cage, in which occur alternate contractions and relaxations of antagonistic groups of muscles. The innervation of these muscles is similar to that of other vertebrate forms, namely, by the afferent and efferent nerves which enter and emerge from the spinal cord at

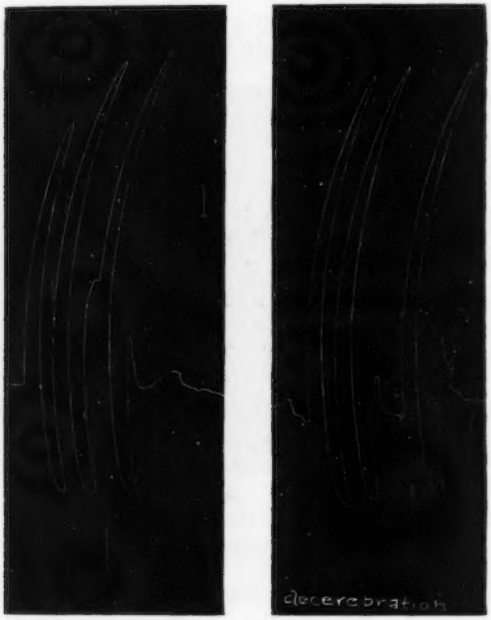

Fig. 2. Tracing 1, Normal respiration. Tracing 2, Respiration after removal of the cerebral hemispheres.

corresponding levels. The respiratory impulse is initiated in the respiratory center of the medulla oblongata and passes by descending tracts in the spinal cord to the motor nerves of the muscles which effect respiration, just as in mammalia.

Previous authors have agreed that so long as the medulla is intact, respiration continues, at slightly varying intervals of time, but if the medulla be separated from the spinal cord, respiration is abolished permanently.

In this study I have endeavored to investigate further,

a. The effect upon respiration of lesions above the medulla.

b. The effect upon respiration of certain lesions of the medulla oblongata.

In these experiments, normal turtles were lightly etherized and a cannula was inserted in the trachea. This was connected by means of double valves (one for intake and one for outlet) with a Verdin recording tambour. Tracings of normal respiratory curves were obtained. The cerebral hemispheres of the animals were then removed and respiratory tracings were again taken (fig. 2).

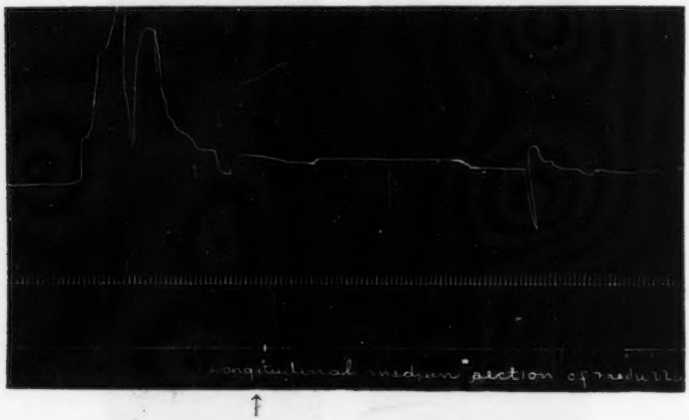

Fig. 3. Respiration after removal of the optic lobes, followed by longitudinal median section of medulla. (Continuation of fig. 2.)

It will be observed from the tracings that the form of the respiratory curves before and after decerebration was the same. The time intervals between respirations which, even under normal conditions were somewhat irregular, varying with the individual animal, were not appreciably altered by decerebration.

On the subsequent removal of the optic lobes, however, the character of the respiration underwent a marked change; the contractions of the respiratory muscles of the thoracic-abdominal cage, which in normal respiration were sudden, sharp and intense, became slow and shallow with greater intervals of time between each two successive respirations (fig. 3).

Following is a procotol of such an experiment.

September 17, 1919

10.00 a.m. Turtle etherized, cannula inserted in trachea and normal respiration recorded.
10.20 a.m. Decerebration; tracing of respiration.
10.36 a.m. Optic lobes removed; tracing of respiration.
11.00 a.m. Section through midline of medulla.

From a number of experiments performed in this manner it has become apparent that a median longitudinal section of the medulla, following removal of the optic lobes, results in an almost complete cessation of respiration. If, however, the optic lobes are intact, a

Fig. 4. Tracing 1, Normal respiration. Tracing 2, Respiration after longitudinal median section of medulla.

longitudinal median section has not such a severe effect. While the form of the respiratory curve may be somewhat altered, respiration proceeds quite adequately.

Several experiments were performed in which the first procedure was a longitudinal median section of the medulla oblongata, with subsequent removal of the optic lobes. Respiration continued until the optic lobes were separated from the brain stem, after which there were few or no respiratory movements (fig. 4).

Normal respiration is not altered by double vagotomy nearly as much as in the case of mammalia. Indeed, the conduct of the respira-

tion seems to indicate that in chelonians the greater part of the afferent impulses originate in the musculature concerned in the maintenance of respiration and are carried by the afferent spinal nerves to the medulla, rather than by the vagi. Moreover, vagotomy does not affect subsequent longitudinal median section of the medulla, as does ablation of the optic lobes (fig. 5).

H. Newell Martin (7) found in the frog, that "when the optic lobes are removed . . . . . such frogs breathe much less often than normal frogs or those with the corpora bigemina intact." From

Fig. 5. Tracing 1, Normal respiration. Tracing 2, Respiration after double vagotomy.

the results of my experiments it appears to be true in the case of the turtle also; and as we ascend the phylogenetic scale, ablation of the optic lobes, or corpora quadrigemina in mammals, is attended by increasingly severe results (8).

In short, when we consider the evidence presented by the conduct of the respiration before and after the removal of the optic lobes, it seems to point to the existence in the optic lobes of nuclei auxiliary to the respiratory center in the medulla for the maintenance of normal respiration.

CONCLUSIONS

1. Stimulation of the optic lobes or medulla produces contractions of the lungs in the turtle.

2. Since after vagotomy, stimulation of the optic lobes or the medulla elicits no contractions of the lung, the impulse to the lungs is carried over the vagus nerve.

3. Since a moderate stimulation of the optic lobes produces only contractions of the lungs and not inhibition of the heart, the nerve fibers in the vagus which pass to the lungs may have an origin anatomically different from those which pass to the heart.

4. Decerebration does not alter respiration in the turtle.

5. From the results obtained by stimulation and ablation of the optic lobes, it may be inferred that they contain nuclei auxiliary to the respiratory center in the medulla oblongata for the maintenance of normal respiration.

I desire to express my thanks to Prof. F. H. Pike of this department for his valuable suggestions and his kindness in criticising this work.

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ON THE PROTECTIVE ACTION OF SOME ORGANIC SUBSTANCES ON CATALASE IN ACID MEDIUM

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The result described here is an objection to Burnett's report (1) which was published last summer. According to his report, the addition of a small amount of liver to muscle increases the catalytic activity of the mass, and blood also has an accelerating effect on the muscle catalase, nearly equal to that of liver. He explained this phenomenon as being due to an internal secretion of the liver, whereby the liver is supposed to send an activator to muscle and other tissues. My experiment has been performed for the purpose of studying *a*, whether his conclusion be true; *b*, how the so-called accelerating effect of the liver changes under various conditions of oxidation in the body and under various conditions of the liver. Satisfactorily as my results agreed with those of Burnett, yet I observed by further investigation that the apparent accelerating effect is chiefly due to the protective action of organic substances in muscle, not to a liver hormone, and what is more, the protective action is by no means specific.

METHOD

As a test object the tissues of guinea pigs were used. The animals were killed and immediately perfused with an m/6 solution of sodium chloride from the aorta, until the fluid coming from the inferior cava was colorless. The leg muscles and other organs were then removed and the water remaining on them absorbed with blotting paper. The tissue and organs to be tested were ground up in a mortar. The pulpy mass thus obtained was weighed and extracted in an ice-chest for 12 hours with four times its volume of m/6 sodium chloride. The mixture was then centrifugalized and employed in volume quantities.

As a substrate 1 and 1.5 per cent solutions of hydrogen peroxide were used, prepared from commercial Sankyo's "Oxyful" with distilled

water, both in neutralized and unneutralized state. The oxygen was collected in a burette over water in the usual way, the amount given off in ten minutes being taken as the standard. The readings were all reduced to 0°C. and 760 mm. Hg. Three tests were made at a time, to avoid the influence of temperature, lighting, etc.

A. Experiment with 1.5 per cent acid hydrogen peroxide, the acidity of which amounts to n/200. In each test 50 cc. of solution were used.

1. The following table shows how the catalytic activity of the mass is increased by the combination of muscle and liver.

TABLE 1

QUANTITY OF EXTRACT		OXYGEN LIBERATED
cc.		cc.
Muscle.....5.0		1.9
Liver.....0.5		13.9
Muscle.....5.0 + liver	0.5	153.1
Muscle.....5.0		1.9
Liver.....0.1		5.7
Muscle.....5.0 + liver	0.1	58.9

2. Table 2 shows the comparison of catalytic activity, when muscle and other tissues were combined.

It may be suggested from the table that the combination of liver and muscle liberated most oxygen, and liver seems to have an accelerating action on the muscle catalase, as supposed by Burnett.

3. To test this supposition, the muscle extract heated for thirty minutes to 100°C. was used after filtration. This solution had no catalytic action on acid or neutral peroxide.

Table 3 demonstrates that the muscle extract, the existing catalase of which was entirely destroyed by heat, liberated, when small quantities of liver, spleen, adrenal gland, etc., were added, as much oxygen as the mixture of fresh muscle extract and other tissues. It is quite evident, therefore, that the liver does not accelerate the muscle catalase, but the accelerating agent is rather contained in muscle itself. As the table shows, the oxygen liberated is just proportional to the catalase content of the organs (cf. tables 2 and 3). These facts prove also that the muscle itself accelerates the catalase of the organs.

4. To test the specificity of the accelerating effect, a combination of liver and other organic substances was made.

TABLE 2

QUANTITY OF EXTRACT		OXYGEN LIBERATED
	cc.	cc.
Muscle	5.0	1.9
Liver	0.5	13.5
Muscle	5.0 + liver 0.5	153.1
Muscle	5.0	1.9
Blood (20 per cent)	0.1	5.6
Muscle	5.0 + blood 0.1	23.5
Muscle	5.0	1.9
Adrenal gland	0.5	8.0
Muscle	5.0 + adrenal gland 0.5	53.5
Muscle	5.0	1.9
Spleen	0.5	0.0
Muscle	5.0 + spleen 0.5	13.5
Muscle	5.0	1.9
Kidney	0.5	0.0
Muscle	5.0 + kidney 0.5	11.2
Muscle	5.0	1.9
Pancreas	0.5	0.0
Muscle	5.0 + pancreas 0.5	4.7
Muscle	5.0	1.9
Testes	0.5	0.0
Muscle	5.0 + testes 0.5	3.8

TABLE 3

QUANTITY OF EXTRACT	BOILED MUSCLE-EXTRACT	OXYGEN LIBERATED
cc.	cc.	cc.
Liver 0.5		13.0
Liver 0.5 +	10	113.1
Adrenal gland 0.5		7.6
Adrenal gland 0.5 +	10	51.9
Spleen 0.5		0.0
Spleen 0.5 +	10	5.9
Pancreas 0.5		0.0
Pancreas 0.5 +	10	3.2
Testes 0.5		0.0
Testes 0.5 +	10	2.7

a. Solution of egg white. Egg white was filtered through gauze and a 10 per cent solution made by adding an m/6 solution of sodium chloride. It was then neutralized with n/5 sulfuric acid. This solution had no catalytic action on acid or neutral hydrogen peroxide.

TABLE 4

QUANTITY OF EXTRACT	SOLUTION OF EGG WHITE	OXYGEN LIBERATED
cc.	*cc.*	*cc.*
Liver.....0.5 +	5.0	125.4
Adrenal gland.....0.5 +	5.0	68.8
Spleen.....0.5 +	5.0	7.0

From table 4 it may be seen that the solution of egg white has also as much accelerating effect as the boiled muscle extract. This action is nearly proportional to the quantity of the solution of egg white.

TABLE 5

QUANTITY OF LIVER-EXTRACT	SOLUTION OF EGG WHITE	OXYGEN LIBERATED
cc.	*cc.*	*cc.*
0.1 +	10	25.0
0.1 +	20	75.0
0.1 +	30	85.0

b. A 15 per cent solution of peptone was heated to 100°C. for thirty minutes and neutralized with n/5 sulfuric acid. This solution had no catalytic action on acid and neutral peroxide.

TABLE 6

QUANTITY OF EXTRACT	SOLUTION OF PEPTONE	OXYGEN LIBERATED
cc.	*cc.*	*cc.*
Liver.....0.5 +	5.0	More than 300.0
Adrenal gland.....0.5 +	5.0	238.7
Kidney.....0.5 +	5.0	52.1
Liver.....0.1 +	5.0	144.1
Adrenal gland.....0.1 +	5.0	44.6
Kidney.....0.1 +	5.0	3.1

It is surprising that the solution of peptone should have an accelerating effect far in excess of other substances!

c. Solutions of starch had no accelerating effect.

B. Experiment with 1 per cent neutral hydrogen peroxide. In each test 70 cc. of solution were used.

In the experiment with neutral hydrogen peroxide it was observed that the accelerating effect is much less manifested, and is sometimes even absent entirely.

TABLE 7

QUANTITY OF EXTRACT	OXYGEN LIBERATED	INCREASE
cc.	*cc.*	*per cent*
Liver.....0.02	22.5	ca. 60
Muscle.....2.00	10.3	
Liver.....0.02 + muscle 2.0	51.7	
Liver.....0.02	29.1	ca. 70
Muscle.....2.00	13.6	
Liver.....0.02 + muscle 2.0	72.0	
Liver.....0.04	89.2	ca. 20
Muscle.....2.0	14.3	
Liver.....0.04 + muscle 2.0	126.7	
	OXYGEN LIBERATED IN 20 MINUTES	
Liver.....0.02 (3 days after preparation)	23.5	Over 100
Blood.....0.02 (10 per cent fresh)	26.3	
Liver.....0.02 (10 + blood fresh) 0.02	125.8	
Liver.....0.05	191.0	Slight retardation
Muscle.....0.00	5.3	
Liver.....0.05 + muscle 1.0	181.0	

These and many other similar experiments with a neutral hydrogen peroxide show the following facts:

1. A combination of any two tissues induces more or less of an increase of the catalytic activity of the mass. This effect is, however, generally much less manifest than when using acid hydrogen peroxide, and sometimes it is entirely absent.

2. The effect of boiled muscle or organ extract and peptone, etc., is similarly diminished when using neutral hydrogen peroxide.

3. The effect seems to disappear at a temperature lower than 14°C.

4. The effect seems to cease in case a relatively large amount of extract is used.

DISCUSSION

The fact that the acceleration is especially manifested in acid medium, while it tends to disappear in a neutral medium, leads us to believe that some organic substance, such as protein, peptone or amino-acid, protects the catalase from destruction by the hydrogen ion in the acid hydrogen peroxide. Whether the mechanism is dependent on neutralization or protecting colloid, is not determined. Loevenhart (2) explained this phenomenon fifteen years ago as being due to a neutralization of acid by some substance contained in the tissue. Burnett contradicted this, however, and maintained that there was a more marked acceleration in neutral peroxide, taking the following example.

1. 0.5 gram of muscle +0.02 gram of liver, acid H_2O_2, gave 170 cc. oxygen.

2. 0.5 gram of muscle + 0.02 gram of liver, neutral H_2O_2, gave 240 cc. oxygen.

This is, however, quite unreasonable. In test 1, with acid peroxide, 0.02 gram of liver and 0.5 gram of muscle give individually a very small amount of oxygen, so he may well say that the liberation of 170 cc. oxygen is marked acceleration. This is not the case with neutral peroxide. According to my experiment, 0.02 gram of liver may give more than 300 cc. oxygen in neutral peroxide, but in acid peroxide only 15 cc. It is therefore necessary to consider the amount of oxygen liberated by liver alone, in order to speak of "acceleration." All in all, there is no discussion about the fact that the apparent accelerating effect is no less than the manifestation of the activity of liver catalase protected by thermo-stable organic substance.

To ascertain the reason for the slight acceleration in neutral H_2O_2 seems to be a rather difficult matter. Since the colloid substance is also increased in this case, the ideal of "protecting colloid" is naturally introduced thereby. On the other hand, the extract itself contains some hydrogen ions, which increase with the autolytic process. These H-ions also destroy the catalase, while the combination may protect it from injury. The addition of fresh blood to a relatively old extract increases therefore the activity most (cf. table 6).

SUMMARY

1. The addition of a small amount of liver to muscle increases the catalytic activity of the mass markedly in acid hydrogen peroxide, while the effect diminishes or disappears in neutral hydrogen peroxide.

2. The accelerating effect is by no means specific. The combination of any two tissues induces more or less of an increase of catalytic activity. And what is more, the boiled muscle and organ extract, the solution of peptone and that of egg white, are as effective as the fresh extract.

3. The increase of catalytic activity is due to the protective action of some organic substance contained in muscle and organ, not to a liver hormone.

4. The mechanism of this protective action is not yet determined, but the idea of a neutralizing or protecting colloid is closely connected therewith.

The writer is indebted to Professor Doctor Mita for his kindly advice throughout the investigation.

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GASTRIN STUDIES

II.[1] Further Studies on the Distribution and Extraction of Gastrin Bodies

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In a previous article (1) the literature on the stimulating effects of organ extracts on gastric secretion was reviewed. It was shown that gastrin is uniformly distributed throughout the stomach; that it is found in much smaller concentrations in the duodenum, and that its presence in the esophagus could just be detected. Preparations of the pancreas, submaxillary gland, smooth muscle, striated muscle gave no gastric stimulation. The brain gave in two instances an increased flow of juice devoid of mucus and acid. The preparations were made by acid (0.4 per cent HCl) digestion of the tissues and the subsequent removal of the proteins by alcoholic precipitation. Popielski (2) contended that secretion in general is a function of the vascular and blood changes which can be brought about by tissue extracts in general. Collectively, he spoke of all of these secretory excitants extracted from tissues as *vasodilatins*. Their solubility in alcohol constituted one of their properties. Therefore, to remove the vasodilating bodies our residues, after the removal of the proteins, were extracted three times with boiling absolute alcohol.

Rogers et al (3) digested tissues in alkaline physiological salt solution. The proteins were removed by heat coagulation in acid solution

[1] Article I—The distribution of gastrin in the body. This Journal, 1915, xxxvii, 481.

The term "gastrin bodies" as used by the authors includes the substance or substances found in tissue extracts which act as gastric secretogogues when injected intramuscularly.

and by salting out with ammonium sulphate. The residue then soluble in 95 per cent alcohol was used as the active preparation. Such preparations of thyroid, liver, parathyroids, spleen and pancreas showed marked activity. The activities of the thymus and pineal gland residues were much lower. Muscle preparations and Witte's peptone were inert. The authors suggested that the gastrin bodies are not confined to the mucosa of the gastro-intestinal tract, but are common to many extracts which influence favorably cellular nutrition and consequently the activity of the gastric epithelial cells, possibly through their nerve supply. They stated further that these stimulating residues acted through the peripheral nervous element upon some gastric secreting mechanism. The experimental work to substantiate this statement consists in the repetition of the experiments on doubly vagotomized animals without a change in results. This, of course, points to the mechanism being peripheral, but it does not necessarily mean that it is nervous.

In a more recent study the authors (4) have shown that the secretory response from thyroid residues can be much reduced by injecting adrenalin, atropin, nicotin and extracts of adrenal glands. Because atropin reduces the flow of juice from injection of thyroid residue and because of the established effect of thyroid extracts on metabolism these authors suggest that with the intermediation of the vagus or secretory nerve impulse the thyroid product increases the metabolism of the gastric epithelium. This view would then indicate that the vagus nerve is essentially trophic in its influence on the gastric cells. There is of course no experimental evidence presented by these observers that their extracts obtained from the thyroid really contained the stimulant to metabolism.

Since in our first studies we discarded the alcoholic extract in which Rogers and associates found their chief activity, it seemed advisable to repeat, with the modification suggested, our experiments on the tissues used in the previous report.

EXPERIMENTAL METHODS

Dogs with either gastric fistulae or Pawlow accessory stomachs were used for assaying the secretory strength of the preparations. In some of the animals either the splanchnic nerves or vagi had been sectioned. Our former experience had shown that the Pawlow stomach is a much less sensitive, but more reliable indicator of the gastrin content of a

tissue. It was hoped that by vagotomizing the animal with a gastric fistula an ideal test stomach could be obtained. This would give us secretion from the whole stomach and avoid the spontaneous secretion apt to be initiated through the vagus. The procedure resulted in a more refractory stomach which was by no means free from spontaneous secretion. Section of the splanchnics made the stomach much more responsive.

Acidities were determined by titration with dimethyl-amido-azobenzene (free acid) and phenolphthalein (total acid). These acidities were calculated to per cent of hydrochloric acid and all the figures given in the paper are thus reported. No attempt was made to measure the peptic activity. The degree of acidity and the quantity of juice are sufficient indicators of the state of the mechanism. Indeed, the concentration of pepsin always falls with an increase in flow of gastric juice although the total units of output are greater. So it alone does not furnish a convenient index of secretion.

One cubic centimeter of the extracts represented, as formerly, 4 to 5 grams of fresh tissues. They were prepared by the method already described (1). However the absolute alcohol extracts as well as the absolute alcohol insoluble residues were assayed as to activities after removing the alcohol and making up to volume in water.

Alcoholic extracts. As soon as the alcoholic extracts, which had been formerly discarded, were examined it was discovered that they were quite active. Indeed, it was further evident that the amount of activity was determined by the time and number of extractions.

This is shown in two protocols of experiments using extracts from stomach and duodenum.

Dog I. Pawlow stomach, August 17, 1916. Injection of stomach extract

TIME	QUANTITY OF JUICE IN CUBIC CENTIMETERS	PER CENT FREE HCl	PER CENT TOTAL HCl
9:25	Dressed		
10:25	0.6	0.00	0.02*
10:35	Injected 1 cc. alcoholic extract of stomach intramuscularly		
11:35	2.1	0.07	0.11
12:35	0.2		
12:35	Injected 1 cc. residue after alcoholic extraction		
1:35	3.5	0.16	0.21
2:35	0.4		

* The results of the titrations are calculated as per cent of hydrochloric acid.

Dog IV. Gastric fistula, vagi crushed one year previously, August 23, 1916. Injection of duodenum extract

TIME	QUANTITY OF JUICE IN CUBIC CENTIMETERS	PER CENT FREE HCl	PER CENT TOTAL HCl
9:00	Dressed		
10:00	3.0	0.19	0.24
10:50	Injected 1 cc. of alcoholic extract of duodenum		
11:50	15.8	0.35	0.40
12:50	0.8	0.31	0.38
12:50	Injected 1 cc. of residue of duodenum after alcoholic extraction		
1:50	3.7	0.08	0.14
2:50	0.6	0.05	0.15

Each of these experiments was confirmed on two other animals, one having a very refractory Heidenhain stomach.

IS PEPTIC DIGESTION A FACTOR IN LIBERATING THE GASTRIN ACTIVITY?

Since appreciable peptic hydrolysis occurs in the preparation of the stomach extracts it was thought that possibly the extraction or liberation of the gastrin activity was facilitated in that case and that possibly peptic digestion of other tissues might yield more active preparations than simple extraction by 0.4 per cent HCl. Accordingly various tissues were finely divided, well mixed and separated into two portions; one to be extracted in the usual way with 0.4 per cent HCl and the other digested for five days at 35° to 40°C. in 0.4 per cent HCl with the addition of toluol and 5 grams of scale pepsin (1:3000 U. S. P.) per 250 to 350 grams fresh tissue. The other steps in the preparation were as previously described. Both were finally treated with alcohol in the usual way, but no extraction with absolute alcohol was carried out. The final solutions represented as before 4 to 5 grams fresh tissue per cubic centimeter.

As a control the amount of activity introduced by the pepsin had, of course, to be determined. Ten grams scale pepsin (1:3000 U. S. P. Armour) were dissolved in 0.4 per cent HCl, shaken with toluol, and digested at 35° to 40°C. for five days with frequent shaking. This was then heated to boiling, filtered and treated in the usual way. The final volumes were so concentrated that 1 cc. of the absolute alcohol soluble fraction was equivalent to 0.55 gram of pepsin and 1 cc. of absolute alcohol insoluble residue equal to 1 gram of the scale pepsin. A protocol attached shows that most of the activity goes into the absolute alcohol soluble fraction, but even this is not large.

Dog I. Pawlow stomach, August 1, 1916

TIME	QUANTITY OF JUICE IN CUBIC CENTIMETERS	PER CENT FREE HCl	TOTAL HCl
10:40	Dressed		
11:40	1.0	0.00	0.03
11:55	Injected absolute alcohol soluble fraction equivalent to 1 gram of scale pepsin (1 to 3000)		
12:55	2.4	0.14	0.17
1:55	0.5		
2:20	Injected absolute alcohol insoluble fraction equivalent to 1 gram of scale pepsin (1 to 3000)		
3:20	1.6		0.03

A more refractory Heidenhain stomach gave just a trace of activity, while dog V (gastric fistula with vagi crushed one year previously) secreted from the entire stomach during the hour following the injection only 9 cc., free acidity 0.24; 0.32 total per cent HCl.

Reference to the long list of inactive preparations in table 4, in which the extracts injected corresponded to 0.01 gram, 0.07 gram and 0.1 gram of scale pepsin respectively, demonstrates clearly that any activity of the tissues extracted by the pepsin treatment is due to substances other than that introduced with the enzyme.

Duodenum. Reference to table 1 shows that when both absolute alcohol soluble and insoluble fractions are injected, the duodenal extract is quite as active as the stomach. The use of the scale pepsin does not appear to affect the activity of the preparation in any constant way; at any rate the comparative study suggests a loss or gain in activity, depending on which animal one considers. In five out of seven cases the stomach preparation was found more active than the duodenal extracts.

TABLE 1

Comparison of activity of gastric and duodenal mucosa

ANIMAL	EXTRACT EQUIVALENT TO 4 TO 5 GRAMS TISSUE	QUANTITY*	ACIDITY	
			Free per cent HCl	Total per cent HCl
		cc.		
Dog I, Pawlow stomach	Stomach	4.9	0.29	0.34
	Duodenum and HCl	3.0	0.17	0.25
	Duodenum and pepsin + HCl	2.5	0.25	0.35
Dog II, Heidenhain stomach	Stomach	1.5	0.19	0.26
	Duodenum and HCl	1.0	0.00	0.05
	Duodenum and pepsin + HCl	1.4	0.09	0.17
Dog III, Gastric fistula	Stomach	7.5	0.33	0.39
	Duodenum and HCl	7.3	0.19	0.34
	Duodenum and pepsin + HCl	12.4	0.39	0.46
Dog IV, Gastric fistula. Vagi crushed 1 year previously	Stomach	9.0	0.37	0.41
	Duodenum and HCl	1.6	0.03	0.08
	Duodenum and pepsin + HCl	6.5	0.22	0.27
Dog V, Gastric fistula. Vagi crushed 1 year previously	Stomach	15.0	0.46	0.50
	Duodenum and HCl	10.5	0.41	0.67
	Duodenum and pepsin + HCl	8.2	0.44	0.50
Dog VI, Gastric fistula. Vagi sectioned	Stomach	11.7	0.15	0.43
	Duodenum and HCl	4.5	0.23	0.42
	Duodenum and pepsin + HCl	15.5	0.14	0.26
Dog VII, Gastric fistula. Vagi sectioned	Stomach	5.2	0.30	0.37
	Duodenum and HCl	6.2	0.26	0.35
	Duodenum and pepsin + HCl	5.0	0.05	0.13

*Quantity represents excess of secretion in experimental over control period.

Liver. The first extract of liver was prepared in August, 1916, and gave activity far above any crude preparations which we have studied. The pepsin digests appeared to be somewht less potent than the simple acid extractions. These points are illustrated in the four protocols attached.

Dog I. Pawlow stomach, September 19, 1916

TIME	QUANTITY OF JUICE IN CUBIC CENTIMETERS	PER CENT FREE HCl	PER CENT TOTAL HCl
7:55	Dressed		
8:55	0.5		0.04
9:05	Injected 1 cc. of liver extract (by HCl)		
10:05	11.0	0.43	0.047
11:05	6.0	0.47	0.51

Dog I. Pawlow stomach, September 21, 1916

TIME	QUANTITY OF JUICE IN CUBIC CENTIMETERS	PER CENT FREE HCl	PER CENT TOTAL HCl
8:20	Dressed		
8:50	0.6		0.09
8:50	Injected 1 cc. of liver extract (by pepsin and HCl)		
9:50	6.5	0.39	0.44
10:50	1.0	0.34	0.40

Dog VI. Vagotomized gastric fistula, September 19, 1916

TIME	QUANTITY OF JUICE IN CUBIC CENTIMETERS	PER CENT FREE HCl	PER CENT TOTAL HCl
8:25	Dressed		
8:55	4.5	0.33	0.47
9:10	Injected 1 cc. of liver extract (by HCl)		
10:10	95.0	0.41	0.49
11:10	6.5	0.33	0.44

Dog VI. Vagotomized gastric fistula, September 21, 1916

TIME	QUANTITY OF JUICE IN CUBIC CENTIMETERS	PER CENT FREE HCl	PER CENT TOTAL HCl
8:35	Dressed		
9:05	8.5	0.36	0.44
9:05	Injected 1 cc. of liver extract (by pepsin and HCl)		
10:05	35.0	0.38	0.46
11:05	4.0	0.29	0.41

These results were confirmed on five other animals in a series of twenty-three experiments. Their uniformity was quite striking. An attempt was made to confirm these results on liver extracts made on October 26 and November 11 of the same year. The first of these stood for two days in the ice-box and the second one was made immediately

upon receipt of fresh liver. On the two preparations of October 26, (one HCl extract, the other HCl and pepsin extract) four experiments were conducted. Reviewing the behavior of these animals we regarded one experiment as positive, two as showing a trace of activity and the fourth negative. The positive experiment is cited to show the low grade of activity as compared with experiment on same animal on September 19.

Dog I. Pawlow stomach, November 9, 1916

TIME	QUANTITY OF JUICE IN CUBIC CENTIMETERS	PER CENT FREE HCl	PER CENT TOTAL HCl
Secretion 1 hr...	1.5		0.08
	Injected 1 cc. of liver extract (by HCl)		
Secretion 1st hr..	2.9	0.09	0.16
Secretion 2nd hr.	1.0	0.14	0.21

On the preparations of November 11, five experiments were run using three Pawlow stomachs, two of these being quite sensitive. Dog IX had both splanchnic nerves cut as they emerge from the thorax beneath the pillars of the diaphragm. The two more sensitive animals responded, but the other one (dog VIII) gave negative results. The activity was somewhat greater than in the extracts of October 26, but was in no way comparable to results on the August preparations.

TABLE 2

Activity of pancreas preparations

ANIMAL	TYPE OF STOMACH	DATE AND METHOD OF EXTRACTION					
		August		October 26		November 26	
		HCl	HCl pepsin	HCl	HCl pepsin	HCl	HCl pepsin
I	Pawlow	−	+	−	+*	−	−
II	Heidenhain	−	+				
III	Gastric fistula	−	+	−	−		
IV	Gastric fistula, vagi crushed 1 year previously	−	+	−	−		
V	Gastric fistula, vagi crushed 1 year previously	?	+	−	?		
VI	Gastric fistula	−	+				
VII	Gastric fistula, vagotomized	−					
VIII	Pawlow			−	−		−
IX	Pawlow, splanchnics cut			−	?		+

Pancreas. Reference to table 2 shows that the August preparation with pepsin extraction was uniformly active on all animals. In only one case (dog V) was there any trace of activity in the HCl extract. The other preparations of October 26 and November 16 gave two positive and two questionable reactions out of seventeen experiments.

An idea of the activity of extracts may be secured from the attached protocols.

August preparations

Dog I. Pawlow stomach, September 24, 1916

TIME	QUANTITY OF JUICE IN CUBIC CENTIMETERS	PER CENT FREE HCl	PER CENT TOTAL HCl
9:50	Dressed		
10:50	1.6	0.00	0.04
11:10	Injected 1 cc. of pancreas extract (by pepsin and HCl)		
12:10	5.7	0.33	0.37
1:10	0.5	0.46	0.55

Dog IV. Gastric fistula, vagi crushed one year previously, September 24, 1916

TIME	QUANTITY OF JUICE IN CUBIC CENTIMETERS	PER CENT FREE HCl	PER CENT TOTAL HCl
9:50	Dressed		
10:50	0.2		0.06
11:10	Injected 1 cc. of pancreas extract (by pepsin and HCl)		
12:10	34.6	0.43	0.47
1:10	4.6	0.40	0.42

With these may be compared the positive experiment on dog I with the preparation of October 26.

Dog I. Pawlow stomach, November 8, 1916

TIME	QUANTITY OF JUICE IN CUBIC CENTIMETERS	PER CENT FREE HCl	PER CENT TOTAL HCl
9:30	Dressed		
10:30	1.6		0.09
10:30	Injected 1 cc. pancreas extract (by pepsin and HCl)		
11:30	2.5	0.09	0.12

The other positive experiment was on the preparation of November 16, the animal used being an extremely sensitive Pawlow stomach, the splanchnic nerves of which had been sectioned.

Thyroid. Consideration of table 3 reveals the fact that thyroid extracts give a rather definite activity by our method. These preparations were made in October under the conditions which had resulted in negative extracts of liver and pancreas. It is further evident that the pepsin does not add to or detract from their activity since in three animals (I, III, VI) the HCl extract was more potent; in two (IV, V) the pepsin and HCl was the more active; in another (VIII) there was no difference; and lastly, dog VII gave results diametrically opposed at various times.

The variability in results is due to the state of the stomach at the time of experiment. As every one knows, this is such a variable factor that the assay of activity frequently requires many repetitions.

TABLE 3

Activity of thyroid extracts

ANIMAL	PREPARATION	INCREASE IN QUANTITY JUICE*	PER CENT ACID IN HOUR FOLLOWING INJECTION	
			Free	Total
		cc.		
Dog I, Pawlow stomach	HCl	3.5	0.18	0.26
	HCl and pepsin	− 0.4	0.13	0.23
Dog III, Gastric fistula	HCl	17.5	0.24	0.29
	HCl and pepsin	1.6	0.02	0.15
Dog IV, Gastric fistula, vagi crushed	HCl	6.5	0.29	0.36
	HCl and pepsin	20.0	0.36	0.42
Dog V, Gastric fistula, vagi crushed	HCl	14.0	0.38	0.42
	HCl	19.0	0.49	0.52
	HCl and pepsin	26.4	0.45	0.50
Dog VI, Gastric fistula, vagotomized	HCl	7.6	0.10	0.12
	HCl and pepsin	− 0.4	0.18	0.27
Dog VII, Vagotomized, gastric fistula	HCl	3.7	0.16	0.24
	HCl and pepsin	− 0.5	0.11	0.22
	HCl and pepsin	7.9	0.40	0.46
Dog VIII, Pawlow	HCl	3.6	0.27	0.34
	HCl and pepsin	3.6	0.20	0.36

* This quantity was determined by subtracting the quantity secreted in 1 hour control period from the first hour experimental period. The (−) sign indicates control output was larger in control than experimental period.

Negative extracts. Table 4 contains a condensed summary of experiments on brain, muscle, spleen, thymus, peptone mixture and gastric juice. The table and the individual protocols show that these preparations do not influence the secretory activity of the stomach.

TABLE 4

Negative extracts and preparations

TISSUE	NUMBER OF EXPERIMENTS	NUMBER OF ANIMALS	NEGATIVE	QUESTIONABLE	POSITIVE
Brain	13	8	10	3	0
Muscle	15	8	15	0	0
Spleen	10	6	7	2	1
Thymus	12	6	12	0	0
Gastric juice	19	8	19	0	0
Fibrin pepsin HCl digest	4	4	4	0	0
Filtrate from the lead acetate precipitate of fibrin pepsin HCl digest	4	4	4	0	0

Gastric juice. The gastric juice was collected under varying conditions of stimulation. Thus samples were obtained from Pawlow stomachs under food and gastrin stimulation, from gastric fistulae under spontaneous secretion and from human stomach (Mr. V. reported by Carlson (5)) following the chewing of food. The juice (1 cc.) was injected without concentration in some cases (3 experiments); in the other cases, the amount injected represented from 4.7 to 11.5 cc. original gastric juice. Animals with gastric fistulae and Pawlow stomachs were used to assay the preparations. In no case was there a positive response.

Fibrin peptone proteose mixture. Popielski (2) has called attention to Witte's peptone as a source from which his vasodilatins could be prepared. It seemed important to investigate this as well as the tissues previously discussed. Instead of starting with a commercial preparation whose history was unknown, a fibrin digest was selected. In this way we could be certain that any activity developing must be due to some of the hydrolytic products and not to substances extraneously introduced. The protocol of this preparation follows.

Beef fibrin was washed free from blood in flowing water, pressed in cloth and the net weight (175 grams) was determined. This was next suspended in 7000 cc. of 0.2 per cent sodium hydroxide for six days, strained and filtered. To the opalescent filtrate was added one volume water and sufficient of a 0.5 per cent acetic acid solution to cause a good flaking out of the fibrin. This latter was allowed to settle, washed

by decantation with distilled water four times, using 8 liters for each washing. Finally, it was filtered by suction and moist weight (206 grams) determined. This constituted the purified fibrin, the substrate from which the peptone was made.

Of this substrate, 103 grams moist weight, (15 grams dry weight) was mixed with 300 cc. of 0.4 per cent HCl and 25 cc. of a 0.1 per cent solution U. S. P. pepsin (Armour's) preserved with toluol and incubated at 35° to 40°C. After three days, 5 cc. more of a 1.66 per cent solution of same pepsin was added; the mixture was incubated another 24 hours, filtered, and the filtrate concentrated in vacuo to 50 cc. The filtrate was then precipitated with 300 cc. of redistilled (95 per cent) alcohol, the precipitate removed and the filtrate again concentrated in vacuo to dryness. Alcohol was removed, the residue dissolved in water and diluted to 25 cc. One cubic centimeter of this solution represented about 4 grams by weight of the fresh material and approximately 0.004 gram of scale pepsin. This constituted the test solution of proteose-peptone which was injected into the animals.

Reference to table 4 shows that the experiments all gave negative results. This solution was further precipitated by basic lead acetate and after the removal of the lead from the filtrate tested against the same animals. This procedure was followed because in the course of studies on purification of gastrin from stomach mucosa the active principle had been found in the filtrate from the basic lead acetate precipitate. These experiments also showed no activity.

We may conclude that an acid pepsin digest of purified fibrin gives no products which are capable of causing gastric secretion when injected intramuscularly.

DISCUSSION

Solubility of gastrin bodies in alcohol. Our experiments demonstrate that the gastrin preparations exhibit rather definite evidence of vasodilatation as shown by the reddening of the nose and buccal mucosa of the experimental animals. We have not taken records of the blood pressure on intravenous injection. However, we published a tracing (1) showing that the blood pressure was not appreciably reduced when the injection was made into the muscles of the animal and we called attention to the fact that the maximum period of secretion came after the evidences of vasodilatation had subsided. In the data just presented it is clear that absolute alcohol cannot be relied upon to separate

the two physiological activities, i.e., the vasodilatation observed after intravenous injection and the secretogogue action resulting from intramuscular injection or, in other words, the vasodilatins of Popielski from the gastrin bodies, if they really be different substances.

Formerly we concluded that the duodenal preparations presented a secretory activity definitely less than similar preparations from the stomach. When however we combined the alcohol soluble fraction, previously discarded as containing vasodilatins, the activity of the two tissues was the same. Maydell (6) states that if secretin is treated with alcohol and ether it is possible to separate the two component parts of gastric secretin, one of which evokes secretion and the other dilates vessels. Up to date we have been unable to secure his original report and for this reason cannot discuss the results further than to quote the words of the abstractor.

Tomaszewski (7) extracted the residue of his preparation (stomach mucosa extract dried at 90°) with absolute alcohol. Practically all of the active substance was removed from the insoluble residue by this treatment, but only about one-seventh of the original activity could be found in the filtrate. A precipitation of proteins by six volumes absolute alcohol on the contrary appeared to increase the activity in the filtrate. This he attributed to a physical change brought about in the mixture, perhaps freeing the body in question more definitely from the proteose-peptone molecules. Again, he precipitated the acid preparation with six volumes of alcohol, the residue was discarded and then a second precipitation was made upon the filtrate after reconcentrating. After three such treatments the activity in the last filtrate was almost completely lost. This experiment only means that the major portion of the active substance must have been carried over in the precipitates and thus disappeared. We conclude that these experiments furnish satisfactory confirmation that gastrin bodies are difficultly soluble in absolute alcohol.

Relation of gastrin bodies to the vasodilatins of Popielski. It is our belief that it cannot be determined at present whether the power of stimulating the gastric mucosa and of causing vasodilatation are two properties of the same substance, or whether they are to be attributed to bodies of different chemical structure. However, Thomaszewski's (7) experiments are of interest at this point. He confirms our former statement that intravenous injections of known active extracts gave little or no secretory response, but a maximum of toxic manifestations. Such a secretion as results he believes is purely a mechanical squeezing out of

residual juice that may have been present. Thus, in addition to his extracts he found that 20 cc. of 5 per cent Witte's peptone solution also gave a small secretion when introduced intravenously, but no secretion at all when injected subcutaneously. Therefore he considers that there are two separate mechanisms involved. The one is elicited by intravenous injections and is perhaps due to mechanical factors, blood alterations and vascular changes. This is the secretion due to vasodilatation and the secretion which he claims will be given by any proteose-peptone solution as, for example, Witte's peptone. The other is elicited on the subcutaneous injection of a body that is a real secretory excitant. Tomaszewski also repeated the experiments of Edkins on cats, but he was able to get only an insignificant increase in acidity of the sodium chloride solution introduced into the stomach. This result he believes is to be explained as a reaction of the first type and that therefore Edkins has in no sense demonstrated the existence of a gastric hormone.

Effect of pepsin on the extraction. It is possible that the pepsin and HCl digestion occurring in the process of preparing the stomach extracts may be a factor in the production of the active substance, either liberating it through the more thorough breakdown of the tissues or through the actual hydrolysis of a particular complex. In other words, the cleavage of the molecule by pepsin may be significantly different from the simple HCl digestion, and to this difference the activity of the preparations may be attributed. However, our experience shows that pepsin does not influence the extraction in any constant fashion. Thus it appears to increase the activity in the cases of duodenum and pancreas, to lessen it in the liver preparations and not to influence it in the case of the thyroid. However, Tomaszewski has published one experiment showing that incubation of an active extract with gastric juice for two days reduces its activity to one-third the value of the original. He concludes that pepsin may destroy this class of bodies, but trypsin does not.

Distribution of gastrin activity. Our results indicate that the stomach, duodenum and thyroid have the same concentration of active substance per gram of fresh tissue.

The liver and pancreas showed activity in one preparation far above any other tissue extracts, but the activity was practically absent in other samples prepared some months later. We are unable to explain this result at present. However, we will have occasion to show later that there are at least two classes of substances which produce secretory

activity. These we know behave differently in their precipitations. Gastrin is extremely stable in acid media, but the stability of the other class of substances has not been investigated. It is possible, therefore, that in the case of the pancreas and liver the activity may be due to this second class of substances. The readiness with which these two tissues autolyze and the ease with which they may be converted into excellent bacterial media may explain the formation of this second group of active substances. Finally, the liver is an extremely mobile metabolic organ and variations in the nutritional state of the animals might explain the discrepancy. Whatever may be the explanation, our method did not give uniformly active preparations from these tissues.

Spleen, thymus, brain, muscle, gastric juice and fibrin peptone-proteose digest were uniformly negative. These results differ from Rogers and associates who found the spleen, pancreas and liver active, with thymus showing a smaller concentration of the active substances. It must be recalled that these investigators used an alkaline physiological salt solution for their extraction while we were using 0.4 per cent HCl. This of course does not mean that bodies possessing secretory activity may not occur in these tissues. It simply indicates that our method which has uniformly given active preparations from stomach and duodenum gives such an active preparation from the thyroid, but not a uniformly active one from liver, pancreas, spleen, thymus, brain, striated muscle, gastric juice and fibrin digest.

It is an interesting question whether the active substance is preformed in the tissues and merely liberated by the acid digestion or whether it is a product resulting from the acid hydrolysis of protein. We have some evidence suggesting the latter to be true. Some of our earliest observations not reported up to present, are to the effect that water extracts of well dried gastric mucous membrane freed from fats are not active as secretogogues. The fact that gastrin is present uniformly in some tissue digests and not in others does not rule out the possibility of its being a building stone in certain proteins. If it be an extractive then its physiological possibilities assume greater interest. The absence of this substance or these substances from the gastric juice should be emphasized. We will have occasion to return to this point in a later communication.

In view of our other experiments attempting to purify the stomach preparation, it seemed inadvisable to pursue further the question of distribution of gastrin bodies until we knew more of their chemical behavior and nature.

SUMMARY

1. Gastrin bodies are soluble in absolute alcohol. Therefore the method of absolute alcohol extraction cannot be used in separating the vasodilatins of Popielski from them.

2. When an extract corresponding to 1 gram of Armour's scale pepsin (1/3000) is injected, a slight secretory activity results. Secretion does not occur after an injection of 0.10 gram pepsin, the quantity used in the peptic digestion of the various tissues.

3. The stomach and duodenum contain approximately the same concentration of gastrin bodies.

4. A liver preparation made at one time gave an activity far in excess of the extracts from any other tissues. Similar extracts, prepared later, were practically inactive.

5. The pancreas extracted with pepsin and hydrochloric acid (on one occasion) gave more activity than the stomach and duodenum, but less than the liver. The hydrochloric acid extract at the same time was inactive. Similar preparations on another occasion (two months later) were inactive. The reason for this difference has not been determined.

6. The thyroid preparations presented about the same order of activity as the stomach and duodenum.

7. The spleen, thymus, brain, muscle, gastric juice and fibrin peptone-proteose preparations were uniformly inactive.

8. Pepsin and hydrochloric acid is not a better medium for extraction than hydrochloric acid alone.

9. The existence of two classes of bodies causing gastric secretion is suggested. Whether these bodies are extractives from special tissues or hydrolytic cleavage products has not been determined. The investigation in the distribution of gastrin bodies has been temporarily abandoned for the more promising studies into the chemical nature of the product derived from the gastric mucosa.

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PHYSICO-CHEMICAL STUDIES ON BIOLUMINESCENCE

I. On the Luciférine and Luciférase of Cypridina hilgendorfii[1]

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INTRODUCTORY

Raphael Dubois (2) found in 1885 that two substances are concerned in light production of the West Indian cucullo, Pyrophorus noctilucus and also of a luminous mollusc, Pholas dactylus. He called one of these light-producing substances "luciférine," which is not destroyed by boiling, and the other "luciférase," which is destroyed by boiling and was assumed to be an oxidizing enzyme.

E. Newton Harvey who investigated the mechanism of light production in luminous bacteria, fire-flies and others, "believed Dubois' interpretation" of these experiments to be correct. But after he studied the light production of a Japanese ostracod crustacean, Cypridina hilgendorfii, he was led "to wholly different conclusions regarding the existence of luciférine and luciférase" (6, p. 322). He has proposed new views concerning the light-producing substances and has adopted new words, "photophelein" and "photogenin" for Dubois' luciférine and luciférase.

The writer has attempted to test Harvey's conclusions with Cypridina hilgendorfii and has found many experimental facts essentially contradictory to those of Harvey and rather in accordance with those of Dubois.

MATERIAL AND COLLECTION

The material used by the writer for all of the following experiments was the same species of ostracod crustacean, Cypridina hilgendorfii[2]

[1] A preliminary report of this work was published in the Japanese Journal of Zoölogy, 1918, xxx, 409, 445.

[2] The writer's thanks are due to Dr. Naohide Yatsu for identification of the animal.

used by Harvey, which is abundant in Tsuyazaki gulf, Japan. It is caught the year round but is most abundant during April and November.

The animal in general is strongly negatively heliotropic, so that it is best collected at night by means of a porcelain jar about 30 cm. high and 20 cm. in diameter. The head of a shark (on which the animal will feed) is placed in the jar, which is covered by a piece of cloth with a small hole in the center. Ten or more of such jars may be used in series connected with a long rope. These jars are submerged to the bottom of the sea about 15 feet deep. The animals go into the jars being attracted by the smell of the fish heads, and stay there feeding. This is a very striking case of chemotropism. In this way large quantities of the animals are readily collected.

Harvey states that the animal is not readily caught on moonlight nights on account of its negative heliotropism. If, however, the method just mentioned above is adopted, no difficulty is encountered even on moonlight nights. The positive chemotropism of the animal is much stronger than its negative heliotropism. Harvey also states that "another non-luminous species (Cypridina x) is often obtained from the fish heads together with C. hilgendorfii. It is positively heliotropic to lamp-light" (6, p. 319). "Non-luminous Cypridina x" is, however, not caught at Tsuyazaki gulf, so far as the writer's experience goes; but some individuals of Cypridina hilgendorfii, and sometimes many of them, are found to be positively heliotropic to strong daylight.

THE MAXILLARY GLAND AND LUMINOUS SECRETION OF CYPRIDINA HILGENDORFII

A full account of the maxillary gland and its luminous secretion will be found in the papers of Harvey (6) and Yatsu (8). It is, however, not out of place to call special attention to the fact that Müller, the discoverer of this species, first pointed out the presence of "two groups of gland cells of different nature" with different secretion products. "Furthermore, he advanced the view that light is produced by the interaction of these two substances" (8, p. 438. Watanabe also states that the maxillary gland secretes a colorless transparent fluid and a yellow homogeneous substance (7, p. 87). Yatsu has especially emphasized these points in his paper based on his histological studies (8), (though Harvey has entirely overlooked them); particularly "the presence of two kinds of gland cells and the absence of a reservoir for the secretion granules common to all the gland cells" (8, p. 438).

Special attention is called to the fact, which has been made clear by the statements referred to above, that the animal produces no light in the maxillary gland cells but the light appears outside of the body of the animal when the light-producing substances secreted by the cells meet in the sea water. In other words, Cypridina hilgendorfii has in no sense any luminous organ or organs as have many other animals, fireflies, for example. In the writer's opinion, therefore, Harvey was entirely misled on this point when he uses such phrases as "the luminous parts" or "non-luminous parts of Cypridina hilgendorfii," "the luminous organs of Cypridina," "the luminous gland" or "luminous gland cells" and so forth all the way through in his paper (6). This point should be remembered when the distribution of the light-producing substances is discussed later on.

THE PRESERVATION OF THE MATERIAL

For various reasons, living Cypridinas are not good material to use for experimental work. The writer, therefore, always used dried animals, except for some special purposes.

Harvey states that he dried Cypridinas over $CaCl_2$ (6, p. 321). But this is an extremely slow process and the light-producing substances are not often in a satisfactory condition, most probably being injured by moisture in the course of the drying time. The writer adopted, therefore, the following rapid method, which proved satisfactory, as table 2 will show. He made a wire ring to which white cloth was fastened. The animals taken out of the sea water were placed on the cloth. The water on the animals was then removed by absorbing it in blotting-paper as completely as possible. After this they were spread on sheets of dry blotting-paper and exposed to direct sunlight. They were stirred up from time to time in order to dry them evenly. At 30 to 35°C., they could be thoroughly dried in a few hours. If the animals thus dried are placed in a desiccator with $CaCl_2$ they may be kept over eight months without impairing their power to produce light when again moistened.

Harvey states that removal of the animals "from sea water also inhibits the ejection of the secretion" (6, p. 320). But this statement is not quite correct. In the course of drying, the animals may live for thirty minutes or longer even in direct sunlight (the time depending upon the temperature) and continue to eject the secretion which is readily observed by the naked eye, as judged by the color. The light-producing substances, therefore, may be lacking in some animals, so

that they may not be of much use when they are finally dried. The quicker the drying, therefore, the better the method, beyond doubt.

The animals dried with the method just mentioned may also be preserved as a whole in pure ether. Or they may be crushed and sifted with a proper sized sieve in order to separate bodies and shells; and then the bodies, either as they are, or after extracting the fatty substances by several changes of ether during the course of a few days, may be preserved in pure ether as experimental material for as long as eight months or more. Of alcohol, ether and chloroform as preservatives, ether is the best owing to its low specific gravity. The material readily sinks into it. This is not the case with chloroform. Moreover, ether is most convenient to work with because of its quick evaporation when the material is taken out of it.

THE SEPARATION OF THE MAXILLARY GLAND

On account of its minute size it is next to impossible, if not absolutely impossible, to dissect out the maxillary gland by itself. At any rate, the writer confesses that he has not succeeded in this attempt. Nevertheless, he believes that the following method is an improvement upon that of Harvey (6, p. 325):

Under a lens the anterior part of dried crushed Cypridina where the maxillary gland is located, is carefully dissected out with one pointed dissecting knife in each hand. But it is hardly necessary to mention that other substances besides the light-producing substances are also cut out. The dissected gland cells examined under a low power of the microscope are very dark, though they are yellow while living. The dissected anterior parts, however, cannot be kept very long in contact with air, though they may be preserved a few days in a vacuum desiccator without impairing the power of light production. The light-producing substances are undoubtedly destroyed by moisture or oxygen or by both.

THE EXISTENCE OF LUCIFÉRINE AND LUCIFÉRASE

As already stated, the existence of two different substances necessary for the production of light by Cypridina was pointed out by Müller, Watanabe and Yatsu on the basis of morphology. Harvey also demonstrated this fact experimentally in Cypridina, as Dubois did in a beetle and a mollusc. Unfortunately, however, the latter

two investigators differ completely in their interpretation, although the results seem essentially similar in nature. At any rate, the fact that the existence of two different substances is concerned in the light production of Cypridina, is readily demonstrated in the following way.

About twenty of the dried individuals are placed in 50 cc. of distilled water. Immediately after this treatment, a brilliant light ensues. After a few hours' standing, the mixture is filtered through heavy filter-paper and the filtrate, which gives no light by itself any longer,[3] is kept for testing. For convenience's sake, this water extract may be called "A" solution. On the other hand, another set of twenty individuals are put into 50 cc. of boiling distilled water.[4] No light results in this case. After a few minutes of boiling, the cooled mixture is filtered and the filtrate (which produces no light by itself) is kept for testing. This may be called "B" solution. If now a small amount of "A" solution is added to a large amount of "B" solution, a brilliant light results at the moment and place of the contact of the two solutions; and the light spreads all over. Its brilliancy does not, however, last very long, but it remains dim for many hours to trained eyes in a dark room.

This phenomenon may be explained on the hypothesis that two light-producing substances are secreted respectively by two different kinds of cells in the maxillary gland. At any rate, that the presence of two different substances is concerned in the production of light by the animal is obvious. That is to say, one of these two substances is destroyed by boiling while the other is not. This thermolabile substance which may be temporarily called X substance, therefore, is left in the "A" solution and the thermostable one, which may be called Y substance, is in the "B" solution intact. The mechanism is this: When the mixture of the material and water is allowed to stand, all the Y substance is used up and the X substance is left in the mixture. On the other hand, when the mixture is boiled the X substance is destroyed while the Y substance is left intact, because the former is destroyed by heat before it can use the latter for light production. On this view, the Y substance is "the source of the light" and the X

[3] Light may be observed by trained eyes even after ten hours, though faint. Harvey might have mistaken it as light produced when this solution was mixed with many chemical substances.

[4] The beaker for boiling water should be tall and the flame has to be lowered after the water has reached the boiling point. No material must stick on the wall of the beaker when the material is put in.

substance is something which causes the former to emit light, as already stated. This conclusion is opposed to that of Harvey (6, p. 323) and is similar theoretically to that of Dubois.

According to Harvey, the substance destroyed by boiling "possesses certain properties . . . . characteristic of enzymes" (6, p. 323). But it can not be regarded as an enzyme because it is "slowly used up" in the reaction (6, p. 324). He, therefore, invented a new word, "photogenin," to indicate the "light producer" for Dubois' luciférase. And this, claimed Harvey, is "the source of the light" (6, p. 323). In the writer's opinion, however, it is hasty to conclude that the substance is "used up" when it slowly disappears in the reaction, because it may not be "used up" in the reaction, but its nature may be changed due to the instability of the enzyme itself. It is a well-known fact that a solution of any enzyme loses its activity if kept. "This is, in great part, due to the complex colloidal state of these substances" (1, p. 313). On this view, then, the disappearance of the thermolabile substance furnishes strong evidence of an enzyme, although Harvey declares to the contrary. The writer, therefore, could find no objection in using Dubois' luciférase to indicate the nature of the action of the thermolabile or X substance found in the gland cells of Cypridina. For this reason he will hereafter use the word "luciférase" to indicate the X substance.

As to the Y substance which is not destroyed by boiling, Harvey names it "photophelein" for Dubois' luciférine, that is the "light assistor" (6, p. 324). This "is something which causes the luciférase to emit light" (6, p. 323). As already pointed out, however, the writer regards this substance as the source of the light, contrary to Harvey's interpretation. The writer therefore defends Dubois' luciférine, as he thinks it adequate to indicate the light-producing nature of the thermostabile substance.

As far as the interpretation of the facts and the phraseology of the light-producing substances are concerned, Dubois and the writer are quite in agreement with each other. According to the former, however, the luciférase of Pholas is an oxidizing enzyme while the luciférine is capable of oxidation, with light production, by means of the luciférase. Since the writer has recently found that the phenomenon of light production of dried crushed Cypridinas is by no means an oxidation,[5] he can neither hold the luciférase of Cypridina as an oxidizing enzyme nor the luciférine as a substance capable of giving

[5] The experimental evidence will be published in a separate paper.

light by oxidation. As Harvey found (6, p. 328), the writer also found that Cypridina luciférine can not be oxidized with light production by such oxidizing agents as neutral H_2O_2, PbO_2, BaO_2, $KMnO_4$ and $K_2Cr_2O_7$, although Dubois found just the contrary in Pholas luciférin. It is possible, however, that the conditions in Pholas dactylus may be radically different from those found in Cypridina. The writer has, therefore, no intention to make any claim against Dubois' conclusion.

THE DISTRIBUTION OF LUCIFÉRINE AND LUCIFÉRASE

According to Dubois, Pholas luciférine exists only in the luminous organs of the animal while the luciférase occurs throughout its body and also in many other non-luminous animals. On the contrary, Harvey finds that Cypridina photogenin (Dubois' luciférase) is only in the luminous organs of the organism, while Cypridina photophelein (Dubois' luciférine) "is found in many other non-luminous animals and in the non-luminous parts of Cypridina hilgendorfii" (6, p. 323).

The writer removed the shells of ten dried Cypridinas and cut them into anterior parts with the maxillary gland cells and posterior parts with no gland cells. Special precaution was taken not to mix one set with the other. The water extract of these posterior parts was mixed with a water extract of dried crushed Cypridinas in which the luciférase was left. This operation was, of course, performed in a dark room. If the luciférine (Harvey's photophelein) existed in these posterior parts, as Harvey claims, the mixture of these two extracts just mentioned should produce light. But this was not the case. The anterior halves with the maxillary gland, however, gave a brilliant light when moistened. On the other hand, the water extract of these posterior parts was mixed with the boiling water extract of dried crushed Cypridinas in which the luciférine was left. If the luciférase (Harvey's photogenin) is present in the posterior parts of the animal, the mixture of these two extracts should produce light. This was not, however, the case. Each of these two series of experiments was repeated several times with no exception. The writer is forced, therefore, to conclude that neither luciférine nor luciférase occurs in the posterior part of dried Cypridina. In other words, the luciférine and luciférase of Cypridina are found in the maxillary gland cells of the animal but in no other part.

There is a possibility, however, that either luciférine or luciférase may exist in other parts of living Cypridina beside the maxillary gland,

even though they occur only in the maxillary gland of the dried animal. In other words, either of these substances might be destroyed by drying, although existing in the living. The fact that both the luciférine and luciférase in the maxillary gland cells of the animal are dried without impairment hardly favors this idea. But the writer repeated Harvey's experiments. One living Cypridina at a time was removed from sea water by means of cloth fixed on wire and was placed on blotting-paper to remove the water adhering to the shell. Of course it was still alive after this treatment. It was then quickly cut by sharp scissors into the anterior and posterior parts. Five of these posterior parts with no maxillary gland cells were mixed with the water extract of these anterior parts with the gland cells or with the water extract of dried crushed Cypridinas. If the luciférine (Harvey's photophelein) were present in the posterior part of the animal, as Harvey claims, the mixture of these two should give light, as the luciférase (Harvey's photogenin) is left in the water extract of these anterior parts or dried Cypridinas allowed to stand. The results were very irregular. Sometimes no light was produced and sometimes one or two of the posterior parts showed dimly lighted points, though they lasted for only a short time. On the other hand, five posterior parts of living Cypridinas were mixed with the boiling water extract of the anterior parts of the living or of the dried crushed Cypridinas. If the luciférase exists in the posterior part of the animal, the mixture of these two should give light, since the luciférine was left in the boiling water extract. The results were again irregular just as those of the other series described above.

If, therefore, one judged from the positive results of these two series of experiments, it might be said that both luciférine and luciférase occur in the posterior part of the animal where no maxillary gland cells are located. On the other hand, if judged from the negative results it is quite logical to say that neither luciférine nor luciférase is found in the posterior part of the animal. The latter alternative seems the truth, because the writer believes that the results ascribed to be "positive" are nothing but false results derived from the rough method of preparing the material for experiment. The difficulty of manipulating the living Cypridina should be taken into serious consideration. Harvey's contention expressed in such statements as, "By a careful quick scissors cut, the head end of Cypridina containing the luminous gland can be separated from the posterior half without any contamina-

tion of the latter with luminous secretion," (6, p. 325), is not beyond criticism.

As already stated, Cypridinas give off the luminous secretion, if they are removed from sea water and are touched with any object, scissors, for instance. It is, therefore, quite possible that the secretion may adhere to the posterior part of the animal. If so, any "positive" results would be deceptive. Unless this objection in preparation of the experimental material is completely wiped out, any positive results are of no value. Even Harvey's statements, if subjected to a careful examination, suggest the possibility of false results, thus: "We must try the experiment immediately because this substance disappears if the extract stands in presence of oxygen. In absence of oxygen or if the extract is boiled immediately (but not too long a time) the substance does not completely disappear even after one hour. There is, therefore, in the non-luminous parts, the substance photophelein which disappears even in the absence of photogenin (from luminous gland) unless the solution be boiled or oxygen excluded" (6, p. 325).

This quick disappearance of photophelein in the absence of photogenin seems to the writer to mean an adherence of a very small amount of the former to the posterior part of the animal.

Furthermore the same "positive" results could sometimes be obtained even in the mixture of the posterior parts with no maxillary gland cells and distilled water. That is to say, the posterior parts of the animal carefully prepared sometimes produced light when mixed with water. Not only that, but a careful examination in a dark room revealed the fact that some of the posterior parts of living Cypridinas dissected out in the same manner as above were already producing light before they were brought in contact with water or any solution—the luciférase solution, for example. These facts undoubtedly prove the adherence of luminous secretion to the posterior parts of the animals, most probably to the shells while handled in cutting.

With the same point in mind, the shells of dried Cypridinas carefully removed for this special purpose were tested with distilled water. Numerous bright small points of light appeared. This fact of course could not be explained unless the light-producing substances secreted are assumed to have adhered to the shells while the animals were handled and dried in the sun.

If Cypridina luciférine occurs in any other parts of the animal where the maxillary gland cells are not found, as Harvey claims, the existence

of two different kinds of gland cells, which is histologically proved, becomes totally meaningless. On the contrary, their existence constitutes very strong evidence of the separate and specific nature of their functions in the production of luminous secretion.

Harvey also claims that he found the photophelein (Dubois' luciférine) in many other non-luminous animals. He has given a list of many animals tested and he adds that "of these forms Lepas and Chiton gave the best light and of these two only Lepas gave light with dilute Cypridina photogenin" (6, p. 326). The writer, therefore, made cold and boiling water extracts of Lepas and Chiton and tested them with extracts of Cypridina luciférine and luciférase. In both cases the results were absolutely negative.

From the variety of evidence, both experimental and histological, considered above, the writer is forced to conclude that Cypridina luciférine and luciférase occur only in the maxillary gland cells of the organism but not in any other part or in other non-luminous animals. In other words Cypridina luciférine and luciférase are specific in the strict sense of the word.

In comparing the results regarding the distribution of light-producing substances in Pholas, Cypridina and other non-luminous animals obtained by Dubois, Harvey and the writer, the following table will assist in visualizing the contradictory points of each investigator's claim discussed above.

TABLE 1

Distribution of the luciférine and luciférase in luminous and non-luminous animals

SPECIES OF ANIMAL	DUBOIS				HARVEY				KANDA			
	Luciférine		Luciférase		Photophelein		Photogenin		Luciférine		Luciférase	
Non-luminous animals	−		+		+		−		−		−	
Luminous animals	In gland	Out gland	In gland	Out gland	In gland	Out gland	In gland	Out gland	In gland	Out gland	In gland	Out gland
Pyrophorus noctilucus and pholasdactylus	+	−	+	+								
Cypridina hilgendorfii					+	+	+	−	+	−	+	−

THE EFFECT OF PRESERVATIVES ON LUCIFÉRINE AND LUCIFÉRASE

As already stated, the writer tested the effect of pure ether, alcohol and chloroform as preservatives of dried Cypridinas. They were preserved in small bottles. Dried whole Cypridinas and dried crushed ones were separately preserved in each substance. From time to time they were tested with distilled water to see whether they would produce light or not. The strength of light produced by them was compared with that of freshly collected and dried Cypridinas as control. The following table will show the results tested after eight months. Of course the preserved animals with the luciférine and luciférase never emit light in these narcotics. The reason is that the luciférine and luciférase are insoluble in the chemicals. They are therefore of a non-lipoid nature. Solubility in water and light production go in union.

TABLE 2

Effect of preservatives on the luciférine and luciférase of Cypridina hilgendorfii

TIME	FRESH CONTROL	PRESERVED CONTROL	ETHER		ALCOHOL		CHLOROFORM	
			Whole	Crushed	Whole	Crushed	Whole	Crushed
Eight months.....	8*	7	6	5	4	3	2	1

* Figures indicate degree of lumination.

The figures in the table indicate the degrees of brightness compared with that of control. The figure "1," for example, means the lowest of all, although it is quite bright. It will be noticed that the brightness of the light produced by dried whole Cypridinas preserved in a desiccator with $CaCl_2$ for eight months is not very different from that of freshly dried crushed ones used for control.

The question arises: if the luciférine and luciférase are insoluble in the preservatives mentioned above, what causes the gradual loss of light-producing power? This is hard to answer. But a few possible explanations may be mentioned. In the first place the preservatives used may not be pure, because they were not purified or redistilled for this purpose. As is well known, the so-called absolute alcohol on the market is about 99.5 per cent at best. Even a little water contained in alcohol might be a cause of slow destruction of the light-producing substances. The same may be said in the case of ether. In

the case of chloroform the material did not sink for a long time due to the former's high specific gravity and perhaps the light-producing substances might be impaired by the moisture or oxygen of the air. The writer is therefore convinced that if extra-pure chemicals are used as preservatives and if the Cypridinas are thoroughly dried, the material may be kept quite long. At any rate the writer concludes that the reason why Cypridina luciférine and luciférase never gave light when mixed with ether, alcohol or chloroform, is their insolubility in these chemicals.

As far as ether is concerned, this conclusion accords with that of Harvey. He says: "Dried crushed Cypridinas may be extracted with six changes of ether during the course of two days without impairing in the least their power to produce light when again moistened. The luminous substance is therefore of a non-lipoid, ether-insoluble nature, as might be expected from the fact that it is extruded from the animal as a clear water-soluble, non-fluorescent secretion" (6, p. 321). He therefore distinctly recognizes the fact of ether-insolubility of the luminous substances, and also the fact of indestructibility of the substances in ether. In other words, he recognizes the fact that no light is produced when both the luminous substances, luciférine and luciférase, are present together in ether. Strangely enough, however, the same investigator states "It (Cypridina luciférase) will also give light if mixed with many pure substances as chloroform, ether, benzol, thymol, saponin, oleic acid, atropin, NaCl and others. Since most of the above substances could not possibly be oxidized by the luciférase, I conclude that they cause in some way the giving out of light in what Dubois terms luciférase" (6, p. 323).

Judging from these statements Harvey seems to identify the action of many pure chemical substances so diverse in nature with that of luciférine. If so, the writer cannot quite understand why he uses the special word "photophelein" (or Dubois' luciférine) to indicate one of two light-producing substances which acts in the same way toward the luciférase (Harvey's photogenin) as chloroform, ether, benzol, thymol, saponin, atropin, pilocarpin, hydrochinon, pyrocatechin, chloral hydrate, butyl alcohol, oleic acid, ortol, aesculin, dextrine, NaCl, $MgSO_4$, $(NH_4)_2SO_4$ or $K_4Fe(CN)_6$, or is omnipresent "throughout the body of Cypridina hilgendorfii" and in "many non-luminous animals." Of course Harvey states, "It is hardly worth inquiring into the nature of the substances in each particular extract which may for convenience be collectively spoken of as photophelein, since

I have found a great many simple bodies which, mixed with concentrated photogenin in powder or crystal form, give rise to a bright light" (6, p. 327). If so, the substance "photophelein," which Harvey thinks he has found in the posterior part of Cypridina hilgendorfii and in many other marine animals, might be NaCl or $MgSO_4$ which are present in sea water in a large amount.

The writer tested Cypridina luciférase solution mixed with pure ether, alcohol, chloroform, NaCl and $MgSO_2$ in various ways to see whether it gives light or not. The results obtained were absolutely negative. Because, as already stated, there is no possibility of light production in the mixture of the luciférase solution and pure ether, alcohol and chloroform, since no light is produced when the luciférine and luciférase are present together in these chemicals. For this reason Harvey's conclusion that the "luciférase is the source of the light and the luciférine. . . . . is something which causes the luciférase to emit light" is not tenable at all.

Although his statements do not make clear how he conducted his tests, Harvey gives two tables in which "the effect of saturation of solutions (one Cypridina to 25 cc.) of photophelein and photogenin with the four substances," that is, chloroform, ether, benzol and thymol, is summarized (6, p. 331). In the first place, however, we must consider from purely physico-chemical viewpoints the properties of these four substances. That is to say, to what extent these substances could be "saturated" with the luciférine and luciférase solutions. Ether is soluble to the extent of 1 part in 12 of water, chloroform to the extent of 0.712 part in 100 parts of water at 17.4°C., benzene to the extent of 0.082 part in 100 parts of water at 22°C. and thymol "is only very sparingly soluble in water." It seemed to the writer to be extremely doubtful that any one could get real results which are to be considered as the effect of saturation of solutions of the luciférine and luciférase with these four chemicals, though Harvey claims that he did.

The writer found that dried crushed Cypridinas produced a brilliant light when they were placed in the saturated water-solutions of ether, chloroform and benzene. No difference of brightness between the light of the animals in the solutions and that of the control in distilled water was perceptible. In other words, the solubility of the luminous substances was not affected by the small amount of ether, chloroform and benzene contained in the saturated solutions. Since neither luciférine nor luciférase when they are present together is destroyed

even by pure ether, chloroform and benzene, as has already been shown (except benzene), it is no wonder that such a small amount of ether, chloroform and benzene as is in the saturated water-solutions of these substances is not detrimental to either luciférine or luciférase. Harvey claims, however, "ether is especially destructive to the photophelein" (6, p. 331). It was not ether, in the writer's opinion, that was especially destructive to the luciférine in Harvey's experiments but it was water. As repeatedly pointed out, even a mere trace of water is destructive to both the luciférine and luciférase.

On the other hand, the writer also tested ether and benzene which had dissolved water to the full extent, that is, to the extent of 2.25 cc. in 100 parts of ether and to the extent of 0.211 cc. in 100 parts of benzene. The results were all negative, that is to say, dried crushed Cypridinas produce no light in ether and benzene thus treated.

It may be worth mentioning that the writer found that living. Cypridinas give light when put in pure ether, alcohol and chloroform This puzzle is readily explained by the following consideration: The living Cypridinas carry sea water inside their shells and secrete the luminous substances when they are placed in the chemical substances: and the secretion meets the sea water carried by themselves. The appearance of light is the result. Neither alcohol nor chloroform plays any rôle in this process.

PROTEIN TESTS ON LUCIFÉRINE AND LUCIFÉRASE

Harvey states that "despite the fact that the light from the natural secretion of Cypridina is very bright, a sample of the secretion, collected by shaking many Cypridinas in a small volume of sea water and filtering, responds to none of the common biochemical tests. . . . . that both Cypridina luciférine and luciférase solutions are positive for proteins. . . . ." (6, p. 322). The writer, however, found that both Cypridina luciférine and luciférase solutions are positive to the biuret, xanthoproteic and other tests. The experiments are in progress and will be given in a separate paper.

THE EFFECT OF TEMPERATURE ON LUCIFÉRINE AND LUCIFÉRASE

Harvey found that "Cypridinas dried over $CaCl_2$, ground, and the powder suspended in sea water give a beautiful light which disappears when heated to 56°, but returns on cooling. If heated to 65° and cooled, the light also returns, but does not return if heated to 70° and

then cooled" (6, p. 329). He also found that "the luminous material of Cypridina. . . . . is unaffected by cold and will glow brilliantly at 0°C." The writer tested the effect of temperature on light production of the animals in a different way from that of Harvey and found practically the same results as the latter. The method will be stated in brief.

The writer heated 40 cc. of distilled water to various temperatures and put twenty dried crushed Cypridinas in it for about thirty seconds (or sometimes for one minute). The water with the animals was then poured into 300 cc. of distilled water which was at about 25°C. In this way the effect of different temperatures upon the light-producing substances was studied, as shown in the accompanying table (table 3).

TABLE 3

Effect of temperature on the luciférine and luciférase of Cypridina hilgendorfii

TEMPERATURE	HARVEY		KANDA	
	Heated	Cooled	Heated	Cooled
°C.				
70	−	−	−	−
69			−	−
67			−	−
65	−	+	−	+
60			−	+
59			+	+
56	−	+	+	+
0	+		+	

SUMMARY AND CONCLUSIONS

1. The animal used for these experiments was Cypridina hilgendorfii.

2. The animal is generally negatively heliotropic, but some individuals of the species are positive to light.

3. The living animal is not good material for experimental work. It is better to use the animal thoroughly dried in sunlight.

4. The dried animal may be preserved in a desiccator with $CaCl_2$ for over eight months apparently without impairing in the least its power to produce light when again moistened. It may also be preserved with some deterioration in pure ether, alcohol or chloroform for over seven months.

5. Ether is the best preservative.

6. The maxillary gland cells of the dried animal are dark red. The red substances produce light when dissolved in water.

7. Water is essential for the production of light.

8. Two substances are concerned in the production of light: the one is destroyed by boiling and the other not. As these two substances show practically the same reaction of luciférine-luciférase as those in Pholas dactylus studied by Dubois, the writer uses Dubois' words, "luciférine" for the thermostable substance and "luciférase" for the thermolabile substance.

9. Harvey's theory of photophelein-photogenin does not fit the author's experimental results.

10. The luciférine and luciférase of Cypridina hilgenforfii are found only in the maxillary gland cells of the animal. Neither luciférine nor luciférase is found in the non-luminous animals.

11. The luciférine does not produce light when mixed with H_2O_2, $KMnO_4$, PbO_2 and so forth.

12. The dried animal does not produce light in pure ether, alcohol and chloroform. The luciférine and luciférase are not soluble in these chemical substances. They are therefore of non-lipoid nature.

13. The living animal may produce light in pure ether, alcohol and chloroform. These chemical substances, however, play no rôle in such production of light. It is due to sea water adhering to the animal.

14. The dried crushed animal gives light in a saturated water solution of ether. This is due to the water, not to the ether.

15. The luciférine and luciférase solutions give the color tests for proteins.

16. The effect of temperature is given in table 3.

The writer concludes that Harvey's new theory and new words are not tenable. The so-called photogenin (Dubois' luciférase) is not the source of the light but may be an enzyme. The photophelein (Dubois' luciférine) is the source of the light.

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PHYSICO-CHEMICAL STUDIES ON BIOLUMINESCENCE

II. The Production of Light by Cypridina hilgendorfii is not an Oxidation[1]

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INTRODUCTORY

That the production of light and oxidation are intimately related to each other is a very general phenomenon judged from common sense and considered from the viewpoints of physics and chemistry. It is, therefore, no wonder that we find in the literature on the subject of the relation of oxygen to light production in living organisms, the statement that the production of light by bacteria, Noctiluca, Pholus, fire-flies and others, is an oxidation (4, p. 353–358).

Harvey (2, p. 321) who has studied the action of oxygen on the production of light by Cypridina hilgendorfii, concludes:

> Oxygen is necessary for light production as may be seen by placing the crushed animals in an hydrogen atmosphere, or by bubbling hydrogen through a glowing extract of the animals. The light never completely disappears even after a long time, but remains dim so that very little oxygen (as no special precautions were taken to remove the last traces of oxygen from the hydrogen, prepared in a Kipp generator) is sufficient to give light. Upon readmitting oxygen, however, a brilliant glow results. Every other species of animal investigated likewise requires oxygen for phosphorescence.

The writer also imagined that "oxygen and water are necessary for the production of light by Cypridina hilgendorfii" and fancied that luciférase "is an oxidizing enzyme" (3, pp. 321 and 448). But the results of his recent work, which was carried out under more careful experimental conditions, have contradicted his expectation. That is to say, he has found that the light production in Cypridina is by no means a phe-

[1] This paper was published in the Japanese Journal of Zoölogy, xxxi.

nomenon of oxidation. Oxygen is not necessary for the production of light by this animal as will be shown in this paper.

The following experiments were conducted in the Science Department of the Kyushu Imperial University, Fukuoka, Japan. The writer expresses his appreciation of the interest and suggestions of Dr. Tsuneya Marusawa, Professor of Physical Chemistry in the University, throughout the course of the work. To Prof. Ayao Kuwaki and all the members of the department, the writer acknowledges his gratitude for the privileges of the laboratory and their interest in the work. The work was supported by the private contribution of Mr. Jihachi Hamano.

MATERIAL

Cypridina hilgendorfii were thoroughly dried in the direct sunlight. The animals were then crushed and the shell and body separated by means of a sieve. The body material containing the maxillary glands was extracted with several changes of ether during the course of a few days "without impairing in the least their power to produce light when again moistened." For convenience's sake, the bodies thus prepared are hereafter called the "experimental material" or simply "material."

DESCRIPTION OF APPARATUS

Believing in Harvey's statements, the writer first tried "by bubbling hydrogen through a glowing extract" or a glowing mixture of distilled water and Cypridinas to see whether the light disappears or not. Hydrogen gas was prepared in a Kipp generator. The method was, however, so crude that he could not obtain decisive results. At the suggestion and also under the direction of Doctor Marusawa, therefore, the writer conducted all his experiments with the following apparatus. The arrangement of the apparatus as shown in figure 1 is typical although it was variously modified for the different gases employed.

The apparatus consists of two wings, right and left. Each wing has an experiment bottle, E or E_1, of a capacity about 60 cc. The bottle is fitted with a tight rubber stopper in which three glass tubes, A, B and C, or A_1, B_1 and C_1, with one stop-cock for each, are inserted with the arm bent at 90 degrees. In the case of the bottle E, the glass tube B with a stop-cock b is connected to a T-shaped glass tube, D, with a stop-cock, d, by means of rubber tube. The second arm of the glass tube D is connected to the flask W by means of glass and rubber tubes,

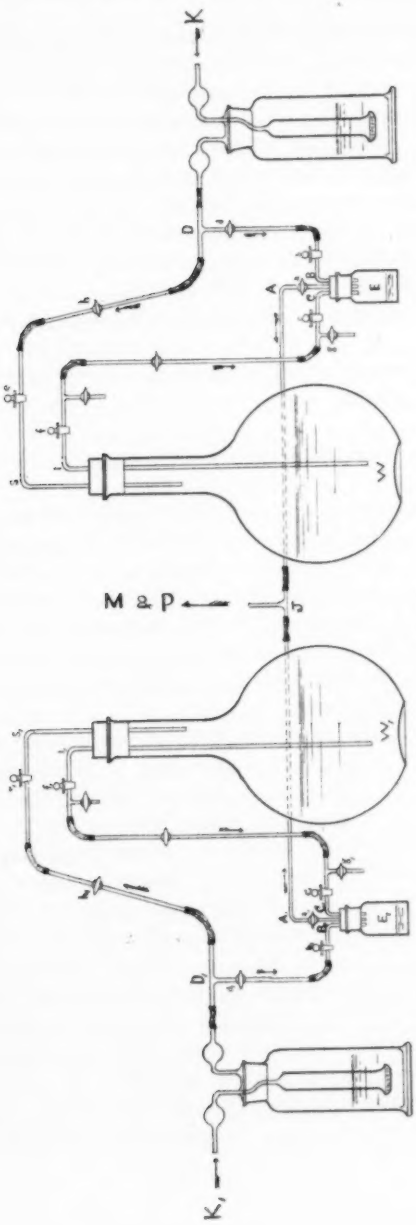

Fig. 1

and the third leads to the Kipp generator, K, through the gas wash bottle. The glass tube C, which has a stop-cock c, and is T-shaped, is connected to the flask W by means of glass and rubber tubes, and a third arm has a stop-cock g by means of which water from the flask W is caused to flow. The glass tube A with a stop-cock a is connected to a T-shaped glass tube J, the second arm of which leads to a Gaede oil pump, P, through a manometer, M, and a safety bottle and the third to the glass tube A_1 of the experiment bottle E_1 at the left wing of the apparatus. The arrangement of the bottle E_1 is exactly similar to that of the bottle E, though sometimes a gas holder is used, depending on the gas under investigation. So the Kipp's generator is not in permanent use.

The apparatus was placed in a dark room, to facilitate the observation of the production of light.

THE METHOD OF EXPERIMENT

As the chief purpose of the following experiments was to determine whether the production of light by Cypridina hilgendorfii is an oxidation or not, a hydrogen experiment was always conducted together with any other gas experiment as a control, besides a control for which air was used. Special care was, of course, exercised when an oxygen experiment was performed. The principle of the method is that the production of light by the material can be observed in the water free from any gases or in any pure gas.

In the first place, therefore, the distilled water to be used should be thoroughly heated by boiling for a few hours. While the water is still boiling the flask W or W_1 is fitted with a tight rubber stopper which carries two glass tubes, long l and short s, tightly fitted. Each of these two tubes has a stop-cock. Steam comes out from the outer ends of these two tubes within a few minutes, since the water is still boiling. These tubes are then closed by the stop-cocks e and f. The glass tube s or s_1, as the case may be, is now ready to be connected to one arm of the glass tube D or D_1 by means of glass and rubber tubes.

One of the most important procedures in this method is to have any desired gas for experiment prepared in the Kipp's generator or the gas holder connected with the gas wash bottles and with the glass tube D or D_1 before the glass tube s or s_1 of the flask W or W_1 is to be connected to one arm of the glass tube D or D_1. In other words, any desired gas should be ready to pass through the gas washers and the glass tube D

or D_1 before the glass tube s or s_1 of the flask W or W_1 is connected to one arm of the latter, and the stop-cock of the glass tube s or s_1 is opened. Because the water and also the space in the flask W or W_1 should be filled up with the desired gas, while the water becomes cooler and cooler. Special care should, of course, be taken that any air in any glass and rubber tubes should be excluded by all possible means, whenever any connection is made. Before the glass tube s or s_1 of the flask W or W_1 is, therefore, to be connected to the tube D or D_1, the washers should be evacuated and then filled with the gas to be used up to the stop-cock h or h_1.

All being thus prepared, the tube s or s_1 is connected to the tube D or D_1 without any unnecessary delay, while the water is still boiling and then the stop-cocks e or e_1, h or h_1 and d or d_1, in order, are opened. Hot steam and gas come out from the end of the arm, because the water is still boiling and the gas is slowly generating. All air is thus driven out from all spaces and the stop-cock d or d_1 is closed. At the same time the flask W or W_1 is removed from the flame. Everything thus arranged, the water and space in the flask W or W_1 are gradually filled up by the gas until the temperature of the water becomes equal to that of the room. When the temperature equilibrium of the inside and outside of the flask W or W_1 has been established, the glass tube l or l_1 is pushed down into the water, and is now ready for its connection to one arm of the glass tube C or C_1 of the experiment bottle E or E_1.

Four bottles, two for the experiment and two for the control, of similar capacity and shape, are cleaned and dried thoroughly. In each of these bottles 0.2 gram of the experimental material is placed. The experiment bottles are then fitted with tight rubber stoppers each of which carries three glass tubes as previously described. One arm of the glass tube C or C_1 which is T-shaped is now connected to the glass tube l or l_1 of the flask W or W_1. And then the gas-saturated water is introduced up to the stop-cock c or c_1, by the management of one arm of the tube C or C_1. The bottle E or E_1 is fixed on an iron stand. Now the connections of the tube B or B_1 through the tube A or A_1 to the T-shaped tube J and the third arm of the last to the pump, P, through the manometer, M, and also a safety bottle, are made by means of heavy-walled rubber tubes. All arrangements thus made, the apparatus is now ready for experiment.[2]

In the first place, the stop-cocks a_1 and b_1 are opened and at the same time the pump is started to evacuate all air in the bottle E_1 and the

[2] It is convenient to begin any experiment from the left wing first.

rest of the apparatus. A complete evacuation of the air, however, is impossible by one operation. In order to complete the evacuation of the air, therefore, it is necessary to fill up all the spaces again with the gas desired, which has been prepared for the experiment. The stop-cock d_1 is now opened, and the gas fills all the spaces. After this filling is complete, the stop-cock d_1 is closed and the pump is again started. This same procedure is repeated ten times, though five times are sufficient. After the last filling with the gas, the stop-cocks a_1 and b_1 are closed. The last procedure is simply to pour a necessary amount of water from the flask W_1 into the bottle E_1 for an observation of the production of light by the material in the bottle. But this should not be done until the bottle E has been prepared in the same way.

To do this the pump P is again started to evacuate the gas in all tubes and spaces from the pump and up to the points of the stop-cocks a and a_1. After this evacuation, the stop-cocks a, b and d are opened. In so doing, another gas is introduced to fill the bottle E and the rest of the apparatus. After this filling the stop-cock d is closed and the pump is again started. After this evacuation, the stop-cock d is opened and fresh gas is permitted to enter. This procedure is repeated ten times as before. After the gas has filled bottle E and the rest of the apparatus, all the stop-cocks, a, b and d, are closed. The bottle E is now also ready to receive water.

Now the stop-cock c is opened and about 20 cc. of the water are poured from the flask W into the bottle E. At the same time, about 20 cc. of the ordinary distilled water are also poured in one of the control bottles which is fitted with a tight stopper. The time should be recorded because it is a very important factor in the production of light. Then the stop-cock c_1 is opened and about 20 cc. of the water are poured from the flask W_1 in the bottle E_1. At the same time the second control is prepared with water. These procedures take about 2 to 3 minutes. Then the bottles E and E_1 with the tubes, A, B and C, and A_1, B_1 and C_1 are freed from all the connections of the rest of the apparatus. At the same time the room is to be darkened. The production of light by the material in the bottles E and E_1 is thus to be observed together. As already stated, special care should be taken that the controls are to be set up at the same time when the water is poured in the bottles E and E_1, since time is a very important factor for the observation of the intensity of the light produced by the material. At the time of pouring water, therefore, at least three persons are to be ready for the work, to save time.

The writer is convinced that the method and apparatus described above are satisfactory to determine the effect of any particular gas on the material.

THE PREPARATION AND USE OF GASES

The gases used for these experiments were hydrogen, oxygen, nitrogen, carbon dioxide and carbon monoxide. Of these gases, hydrogen, nitrogen, carbon dioxide and carbon monoxide were prepared in the laboratory, and oxygen in bomb was used. The methods of preparing these four gases were those in common use. The writer thinks, however, that brief statements about the methods of preparing them may not be out of place, because all the procedures carried out by the writer need to be open for free discussion.

1. The preparation of hydrogen: Hydrogen was prepared with a Kipp's apparatus in which zinc and about 50 per cent H_2SO_4 were placed. The liberated gas was washed by passing it through four wash bottles. Distilled water was placed in the first, and saturated $KMNO_4$ solution in the second, a solution of 30 grams of KOH + 10 grams of $C_6H_3(OH)_3$ in 100 cc. of distilled water in the third, and concentrated H_2SO_4 in the last. As hydrogen was always used as a control in order to compare the action of this gas with that of any other gas, this Kipp's apparatus was always fixed in the right wing. The last wash bottle of this apparatus, therefore, was always connected to the tube, *D*. The solutions in the wash bottles were renewed from time to time.

2. The preparation of carbon dioxide: Carbon dioxode was also prepared with a Kipp's generator in which pieces of marble and about 15 per cent HCl were placed. The gas when liberated was washed by passing it through three wash bottles. Distilled water was placed in the first and second and concentrated H_2SO_4 in the third. This last wash bottle was connected to the tube, D_1, when used for experiment.

3. The preparation of nitrogen: In the first place, 200 grams of $NaNO_2$, 300 grams of $(NH_4)_2SO_4$ and 200 grams of K_2CrO_4 were barely dissolved in separate beakers. The solutions were placed together in a 5-liter flask and 1500 cc. of distilled water were added to it. A condensor was connected to the stopper of the flask in order to cool the nitrogen gas. To this condenser, a heavy Erlenmeyer flask as a safety bottle and two wash bottles were connected. In each of these bottles 30 cc. of concentrated H_2SO_4, 20 grams of $K_2Cr_2O_7$ and 100 cc. of distilled water were placed.

The mixture contained in the flask was slowly heated on a low flame. When enough N_2 gas was liberated, the last wash bottle was connected to a gas holder which was already prepared to receive the gas. Special care was taken to have no air in any space. When the gas in the gas holder was used for experiment, it was again washed by passing through the same wash bottles as mentioned above.

4. The preparation of carbon monoxide: In a liter distillation flask, 500 cc. of 80 per cent H_2SO_4 were placed. In the rubber stopper of this flask, one long thermometer of 200°C. and one glass tube perforated on its sealed end of about 5 cm. with many small holes were inserted. The mercury part of the thermometer and the hole part of the tube were entirely dipped into the sulphuric acid of the flask. The outer end of the tube was bent about 90 degrees and a suitable rubber tube with a pinch-cock was connected to it. The other end of the rubber tube was then connected to a separating funnel in which some formic acid was placed. To the distillation flask, a heavy Erlenmeyer flask as a safety bottle, and two wash bottles, in each of which 200 cc. of 20 per cent NaOH were placed, were connected. The sulphuric acid of the flask was slowly heated on a low flame to about 110°C., and the formic acid was added little by little. After enough gas was liberated, the gas was received in a gas holder.

When the carbon monoxide gas was used for experiment, it was again washed by passing through three gas wash bottles. Two hundred cubic centimeters of 20 per cent NaOH were placed in the first and second bottles, and 200 cc. of concentrated H_2SO_4 in the third, which was connected to the tube, D_1.

EXPERIMENTAL

With the methods and apparatus described in the previous section, the writer conducted his experiments with every possible care and repeated each series of experiments four or five times, even though no exception was found in any trial. The results of these experiments are summarized in table 1.

The figures of the table show a relative superiority and inferiority of the intensity[3] of light produced by the material on which five independ-

[3] The observation of the intensity of light was made at the moment when the two experimental and two control bottles held together in both hands were given an equal shaking. A question may arise about the intensity of light, because the writer has not determined it by a quantitative method. According to the calcu-

ent gases and air were allowed to act. The figure "6," for example, means the highest degree of light intensity compared with all others. As repeatedly stated, the experiments on the action of hydrogen and of air were always made as controls of any other gases. But as the experiments on the other gases, carbon dioxide and carbon monoxide, for instance, were not carried out together, it was impossible to compare their light intensities at the same time. The time for observing the light intensity in each gas, however, was carefully recorded in comparison with that of light produced in hydrogen and in air. In this roundabout way, therefore, the action of each gas may be compared. Furthermore, it was enough if the actions of hydrogen and oxygen were

TABLE 1

Comparative intensity of light produced by the material in various gas atmospheres and water

TIME OBSERVED IN DARK ROOM	LIGHT INTENSITY OF THE MATERIAL OBSERVED AT A GIVEN TIME IN THE ATMOSPHERES AND WATER OF THE FOLLOWING					
	H_2 (Special control)	N_2	CO	CO_2	Air	O_2
1 m.	6	6	6	6	6	6
3–4 m.	6	6	6	5	4	4
5–8 m.	6	6	5	5	3	3
12–15 m.	6	5	4	4	3	2
20 m.	6	5	4	4	2	1
9–10 h.	5	4	3	3	1	0?
13 h.	4	3	2	3?	1	
160 h.	1	1	0	0	1?	
200 h.	0?	0			0	

accurately compared, since the essential problem of these experiments was to determine whether the production of light by the animal was an oxidation or not. There was an unmistakable difference of the light intensity between the actions of oxygen and air. The difference of the intensity of light produced in the hydrogen and oxygen atmospheres, as well as in the air, was so astonishingly marked that no one could

lation of V. Henri et Larguier des Bancels, however, the retina is very "sensitive to an amount of light energy as small as 5 times 10^{-12} ergs. This is about three thousand times as sensitive as the most rapid photographic plate" (1, p. 512). If so, although there is no quantitative means to decide the weak or strong intensity of light observed by the retina, there is no indicator superior to the qualitative judgment of the retina. It is believed, therefore, that the decision made by the retina is most accurate.

question it. And the action of the nitrogen gas in comparison with the action of carbon dioxide or of carbon monoxide was also very distinct. Superiority or inferiority between the actions of CO and CO_2 may be questioned, though the writer felt that the former was a little superior to the latter.

It will be worth while to describe some other facts than those shown in table 1. In the water saturated with air and other gases, the color of light produced by the material is bluish white, a few hours after the treatment, while in the water saturated with hydrogen, *a*, it is decidedly blue. This blue color in the latter lasts almost as long as the light continues. In the cases of air and other gases the light after shaking takes about three or four seconds to return to the state before shaking, while *b*, in the case of hydrogen it takes about eight seconds. In a perfectly dark room it is observed that the strong light after shaking lasts quite long. As the time ratio of durability, 1: 2, however, is not altered, no deviation is involved in this observation. Generally speaking, the "steady homogeneous glow" of light is to be observed, as Harvey pointed out. But *c*, the light in the hydrogen-saturated water is observed to be heterogeneous by the flowing and precipitating of extremely minute particles when shaken in a dark room. Harvey's claim of "complete proof of the truly soluble character of the light-producing substances" (2, p. 321) may not be true. The writer will try to make this point clear by using an ultramicroscope in the near future. In the resting state, *d*, the light in the case of hydrogen glows as if the solution glows itself but the glow of the individual points of the material is not so marked as it is in the cases of air and any other gases, while in the latter cases the solution is clear. And *e*, in the latter cases also the solution forms when shaken but not so much as in the former. In the cases of all gases, when the production of light is near to the end, the solution does not glow as a whole but only at the surface when shaken. This *f*, is markedly so in the case of hydrogen. But in the case of air, the solution glows as a whole till the end.

These are six characteristics of the effect of the hydrogen gas on the production of light by the material besides those shown in table 1. Whether these are of any significance regarding the light production or are simply to be overlooked as meaningless, can not be settled unless further facts are found.

After an experiment was over, a test was made with a lighted match to see whether the gas used was present or not. The results were always positive even after seventy-five days had passed. Each solution

was also tested with litmus paper to see whether it was alkaline or acid. In the cases of hydrogen, nitrogen, oxygen and air, the solution was found to be neutral, although the solution was slightly alkaline after about two hundred hours in the last case. In the case of carbon dioxide, the solution was always acid and was distinctly milky with white precipitation. In the case of carbon monoxide, the solution was also acid, though very faint; and the acidity seemed to increase a little after about two hundred hours. In the cases of oxygen, air and carbon monoxide, the solution and material became very black, while in the case of hydrogen and nitrogen the solution was clear and the material became reddish brown.

CONCLUSION AND DISCUSSION

That the less the quantity of oxygen contained in the experiment bottle the more intense is the light produced by the material after the first minute is quite obvious according to the results stated above. The light produced is more intense in the water saturated with air than in the water saturated with oxygen and is markedly more intense and durable in the water saturated with hydrogen or nitrogen than in the water saturated with oxygen or air. In other words, oxygen is not necessary for the production of light by the material. If so, there is no ground for the assumption that the production of light by the animal is an oxidation. If the production of light by the animal is due to an oxidation, as Harvey claims, the more intense light should be produced by the greater concentration of oxygen. This is not the case, and the writer therefore concludes that the production of light by the animal is not an oxidation.

A question arises whether the production of light by the material is a reduction or simply an hydrolysis. In expectation of getting some light on this question, the writer made experiments with carbon monoxide as a reducing substance. But the results were not so marked as expected. That is to say, the results were not as good as those in the case of hydrogen. If so, is this not a reduction? This question is not settled as yet. Carbon monoxide is a "poison gas." It may, therefore, act as a poison just as it acts on the hemoglobin of the blood. At any rate, it may be necessary to take into such biological consideration some factors besides the action of carbon monoxide purely looked upon from the viewpoint of chemistry. If so, it is no wonder that the action of carbon monoxide on the production of light by the material is inferior

to that of hydrogen or nitrogen. Whatever the exact interpretation of the facts may be, no decisive conclusion can be made unless further facts are found.

As to the action of carbon dioxide, there is the same question as in the case of carbon monoxide. If the light production of the material is not an oxidation, the intensity of light produced should be the same in carbon dioxide as in hydrogen or nitrogen. But this is not the case. However, if the fact is considered that carbon dioxide is dissolved in water and carbonic acid is formed, the riddle may be readily solved. Because it is a well-known fact that acid is injurious to organisms while alkali is not.

Furthermore, there are other points in table 1 which require explanation. In the first place, the light-producing substances in the water saturated with oxygen disappear fastest in spite of the production of the poorest light. On the other hand, the production of light in the water saturated with hydrogen is most intense and most enduring. Such phenomena may be explained by the following assumptions. That the higher the oxygen tension is the faster the oxidation of any substance is obvious. Although the production of light in question is by no means an oxidation, the light-producing substances, especially the luciférine, may always be subjected to an oxidation and thus the disappearance of the substance is apparent. If it be true that the higher the oxygen tension is the more the light-producing substance or substances may be oxidized (though this oxidation of the substances has no bearing on the light production), it is no wonder that the light becomes weaker and disappears faster if the substance or substances become less and less by an oxidation. Such a consideration explains the facts that the light is weakest and lasts shortest in the water saturated with oxygen and on the other hand, that the light is strongest and lasts longest in the water saturated with hydrogen with no oxygen.

If so, the action of the water saturated with carbon monoxide, carbon dioxide and nitrogen might be the same as the action of the water saturated with hydrogen. But this is not the case. As to the action of the first two gases there may be possibly some physiological or biological factors involved, as already stated. So the mere fact that free oxygen is not present should not be looked upon as a complete explanation. Taking this as granted, then, how is it in the case of nitrogen? The difference in action of hydrogen and nitrogen is not very marked, but it is still easily distinguishable. It may be said, therefore, that the superiority of the action of hydrogen over any other gas is its own speci-

ficity. In brief, the writer should confess that he has not yet clear knowledge with which he can explain these various difficulties. He expects to make special efforts to gather all possible data based on experiments. These difficulties may be of such a nature that they may be explained when further facts are available.

SUMMARY

1. The intensity of light produced by the material is strongest and lasts longest in the water saturated with hydrogen.

2. The intensity of light produced by the material is weakest and lasts shortest in the water saturated with oxygen. Therefore the production of light by the material is not an oxidation.

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THE PREPARATION OF ADENINE NUCLEOTIDE BY HYDROLYSIS OF YEAST NUCLEIC ACID WITH AMMONIA[1]

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When yeast nucleic acid is heated in an autoclave with ammonia for several hours at 145°–148°, it completely loses its phosphoric acid so that its four nucleotides are formed (1), (2).

But when yeast nucleic acid is similarly treated with ammonia at a lower temperature (105°–120°) it does not lose any of its phosphoric acid but decomposes into nucleotides (3).

After hydrolysis at the lower temperature, if one will treat the product alkaline as it is, with an equal volume of alcohol, a very useful separation occurs. The guanine complex is precipitated while the adenine complex remains in solution (3), so that the material can be separated sharply into two fractions. This is the analytical key to the separation of adenine nucleotide from guanine nucleotide and without it the results discussed below could not have been obtained. It is based upon the fact that alkaline salts of adenine nucleotide are quite soluble in 50 per cent alcohol while the alkaline salts of guanine nucleotide are practically insoluble (4).

From the *adenine fraction* Jones and Germann (3) prepared the free nucleotide by means of its lead salt and found that it contained a uracil group in addition to the adenine group. From the fact that precisely half of its phosphoric acid is "easily split" and half is "firmly bound" Jones and Read (5) concluded that the substance contains its adenine and uracil groups in equivalent quantities, and is therefore adenine-uracil di-nucleotide. This conclusion appeared to be much strengthened by the following experiment which Jones and Read did not publish. An attempt was made to obtain uracil nucleotide after destroying the

[1] While this article was in press, the contribution of Levene appeared. Journ. Biol. Chem., 1919, xl, 415.

adenine nucleotide with boiling mineral acid. One hundred grams of the supposed di-nucleotide were used for this purpose but 98 grams were destroyed leaving only 2 grams of undecomposed nucleotide material. This was found to have all the properties of adenine-uracil di-nucleotide including the formation of a crystalline brucine salt having the required chemical composition. Thus it appeared that the adenine- and uracil-nucleotides were not only present in equal quantities, but were destroyed in equal quantities.

Jones and Read (6) prepared from the di-nucleotide a crystalline brucine salt which had sharply the composition required for the brucine salt of adenine-uracil di-nucleotide.[2]

Levene (7) prepared this nucleotide by the method of Jones and Germann and converted it into its brucine salt as described by Jones and Read. He states that the brucine salt which he obtained possessed the analytical values required for adenine-uracil di-nucleotide, but found that these values are disturbed when the substance is repeatedly recrystallized from 35 per cent alcohol. After nine successive crystallizations, the surviving brucine salt had a chemical composition very close to that required for the brucine salt of uracil nucleotide. From this brucine salt a crystalline barium salt was prepared and found identical with the barium salt of uracil nucleotide that Levene (8) had already prepared from another source.

Levene's results appeared very convincing to us in so far as they went to show that the supposed adenine-uracil di-nucleotide is in reality a mechanical mixture of its component mono-nucleotides, and his results also suggested to us the possibility of preparing adenine nucleotide from the mixture; for Jones and Kennedy (9) had prepared adenine nucleotide and had found its properties such as should make its isolation from almost any mixture a comparatively easy matter. By the application of their method to this problem, we have isolated pure crystalline adenine nucleotide identical with the substance described by Jones and Kennedy.

Thirty-eight grams of nucleotide were dissolved in 152 cc. of hot water and treated with a solution of 95 grams of brucine in the smallest possible amount of alcohol. On cooling, the material stiffened to a paste of crystals which was filtered on a Buchner funnel and crystallized from 35 per cent alcohol. Sixty grams of this brucine salt were treated

[2] This can be accurately determined because adenine nucleotide and uracil nucleotide differ from each other markedly in composition. The one contains five atoms of nitrogen; the other, only two.

with 6 liters of cold 35 per cent alcohol and after digestion with occasional agitation for 24 hours, the alcoholic extract was filtered from the undissolved residue on a Buchner funnel and allowed to evaporate for several days in open dishes at the room temperature. As the alcohol escaped, a crystalline brucine salt was deposited which was collected, allowed to dry in the air and used for the preparation of adenine nucleotide as described below (25 grams).

The undissolved residue from the alcoholic extraction was again extracted with 600 cc. of cold 35 per cent alcohol and the dissolved brucine salt was recovered after evaporation at the room temperature. This had very nearly the composition of the brucine salt of uracil nucleotide.

0.6136 required 12.04 cc. of standard H_2SO_4 (1 cc. = 0.003642 N).

	REQUIRED FOR		FOUND
	Adenine nucleotide	Uracil nucleotide	
N.....	10.00	6.79	7.15

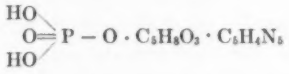
Thus the original extraction with 6 liters of cold alcohol had almost completely removed the brucine salt of adenine nucleotide, but of course the extract contained also a considerable amount of the brucine salt of uracil nucleotide.

Twenty-five grams of the brucine salt mixture (from the 6 L of 35 per cent alcohol) were suspended in 800 cc. of boiling water and treated with a little ammonia which takes it promptly into solution. As the solution cooled, it was made and kept faintly but distinctly alkaline with ammonia. In this way the brucine is thrown down in easily filterable crystalline needles. The material was kept overnight in the ice chest, filtered from the brucine with a pump and shaken in a separating funnel with several successive portions of chloroform to remove the last traces of brucine. The solution was then evaporated[3] at 45° under diminished pressure to one-third of its volume and treated in the warm with a slight excess of lead acetate. The heavy granular lead compound was filtered on a Buchner and washed by grinding with hot water in a porcelain dish. Finally the material was made into a smooth suspension with warm water, decomposed in the warm with sulphuretted

[3] The neutralization of the ammonia with acetic acid as done by Jones and Kennedy should be omitted since the lead salt of adenine nucleotide is quite soluble in ammonium acetate.

hydrogen and the solution obtained after filtering off the lead sulphide was aspirated to remove the excess of sulphuretted hydrogen. On standing over night this solution deposited snow white adenine nucleotide in characteristic needles. The mother liquor from these crystals, upon evaporation at 45° under diminished pressure gave a further yield of the same crystalline material. Altogether 3.12 grams of adenine nucleotide were obtained.

In its chemical composition, crystalline form and solubilities, in its characteristic ability to form supersaturated solutions, in the curious behavior of its water of crystallization and in all other respects the substance is identical with the adenine nucleotide of Jones and Kennedy.



I. 0.3108 required 16.2 cc. of standard H_2SO_4. (1 cc. = 0.003642 N).

II. 0.2374 required 12.32 cc. of standard H_2SO_4.

III. 0.4615 after complete burning gave 0.3105 Mg $NH_4PO_4 6H_2O$.

IV. 0.4335 after complete burning gave 0.2892 Mg $NH_4PO_4.6H_2O$.

V. 0.3416 heated 2 hours with 5 per cent H_2SO_4 gave 0.2292 $MgNH_4PO_4.6H_2O$ and 0.3428 adenine picrate.

VI. 0.3001 heated 2 hours with 5 per cent sulfuric acid gave 0.1982 $MgNH_4PO_4.6H_2O$ and 0.2994 adenine picrate.

VII. 0.5349 lost 0.0262 at 127°.

VIII. 0.3162 lost 0.0149 at 127°.

	REQUIRED FOR $C_{10}H_{14}N_5PO_7H_2O$	FOUND							
		I	II	III	IV	V	VI	VII	VIII
Nitrogen	19.18	19.00	19.02						
Total phosphorus	8.49			8.51	8.44				
Partial phosphorus	8.49					8.48	8.36		
Adenine	37.00					37.20	36.92		
Water	4.93							4.89	4.71

The mother liquor from adenine nucleotide of course contained uracil nucleotide. It was allowed to evaporate at the room temperature to about one-fifth of its volume, filtered from a trace, heated and neutralized with brucine in substance. On cooling the deposited crystalline brucine salt was filtered off and recrystallized from 35 per cent alcohol (6.46 grams); 0.6841 required 13.12 cc. of standard acid (1 cc=0.003642N).

NITROGEN REQUIRED FOR BRUCINE SALT OF		FOUND
Adenine nucleotide $C_{10}H_{14}N_5(C_{23}H_{22}N_2O_4)_27H_2O$	Uracil nucleotide $C_9H_{13}N_2PO_9(C_{23}H_{26}N_2O_4)_27H_2O$	
9.76	6.79	6.99

It seems a little curious that crystalline insoluble adenine nucleotide never appears until one has prepared a brucine salt of this material. The identical procedure described in this paper for preparing the substance from a mixture of brucine salts had been already executed in preparing the original nucleotide mixture, yet this nucleotide mixture could be evaporated to a syrup without depositing any adenine nucleotide. It might be supposed that an impurity in the nucleotide mixture which prevents the crystallization of adenine nucleotide, is gotten rid of in the mother liquor from the brucine salts. But we were able to prepare crystalline adenine nucleotide from this mother liquor just as from the crystallized brucine salts. Perhaps it is necessary to start with a mixture of the nucleotides, in which adenine nucleotide is in considerable excess.

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CHANGES IN THE HYDROGEN ION CONCENTRATION OF THE URINE, AS RESULT OF WORK AND HEAT

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INTRODUCTION

In a previous article (1) I reported on changes in the hydrogen ion concentration of the sweat caused by muscular exercise and heat. At the same time that I was making these determinations I was collecting samples of urine from the subjects in order to ascertain what changes, if any, might be effected in its reaction under precisely like conditions.

Study of the sweat meant working in a quite unexplored field, and this, in part at least, might be said of the experiments that I have to report on the urine, for so far as I have been able to ascertain the effects of heat on the hydrogen ion concentration of the urine have not heretofore appeared in the literature. As to the effects of muscular exercise upon changes in the acidity of the urine, several observers have published their results from time to time. But the fact that the methods that were employed in obtaining the total acidity of urine and similarly constituted fluids are now known to be obsolete, and furthermore that the results reported were somewhat conflicting, suggested a reëxamination of the subject.

The principal observers who have investigated this phase of the problem are Hoffman (2), Ringstedt (3), Oddi and Tarulli (4), Vozarik (6) and Aducco (5). All except the last named have maintained that muscular exercise increased the acidity of the urine; Aducco on the other hand has claimed a decrease in the acidity.

It is well to state at this juncture that in my experiments the subjects were not required to take extremely fatiguing exercise. The work for the time was hard and the heat endured was fairly intense. The subjects remained with me about one hour, which included the time of stripping and dressing.

Exactly the same methods were employed as were used with the sweat, and like precautions were here observed. It seems only necessary to repeat that the hydrogen ion concentrations were obtained by Henderson and Palmer's (7) colorimetric method controlled by occasional electro-metric measurements (Clarke's (8) technique).

EXPERIMENTS

As in the case of sweat, my first observations were upon subjects from the Baltimore Central Y. M. C. A. The exercise taken was basket ball, volley ball and the usual work of a gymnasium, which was generally followed by running, these exercises lasting from one-half to one and one-half hours.

It soon became apparent that volunteer subjects in a large city gymnasium were not altogether dependable; consequently it was found much more desirable to have paid subjects come to the laboratory where their part of the work would come under closer scrutiny.

As these experiments were performed at least two hours, and usually five hours, after eating, and as the intervals between taking of the samples were so short, it did not seem necessary to lay much stress on the diet, particularly as it was the relative rather than the absolute values which were most desired.

I took note on all morning experiments when I discovered that the urine had not been voided since breakfast, especially when alkali-producing fruits had been eaten. The main purpose I had in view was to ascertain whether the effects of such diets might be observed in the absolute values. Some of the high alkaline findings may be due to the retention of the morning urine after eating the above type of fruits. On the whole, in my eighty-six observations of normal urines I found the fluctuating values which have been emphasized by many others.

When the experiments were taken up in the laboratory, a stationary bicycle was employed for the muscular exercise and the sweat-cabinet for the heat. In this connection, however, I might say that heat was not introduced into the experiments with the thought of studying its effect on the urine, but only for the purpose of obtaining heat sweat. It was accidentally and subsequently that I conceived the idea of studying the reaction of the urine under the influence of heat. Consequently, in my first experiments I took only a control sample of the urine and another after the heat sweat had been produced and the muscular exercise had been completed. But after performing three

experiments of this character I decided to test the urine as well as the sweat secreted after heat. So thereafter four samples of urine were obtained at about fifteen-minute intervals. The first sample was taken just before the subject entered the sweat-cabinet, the second just as he came out and was ready to exercise on the bicycle, the third imme-

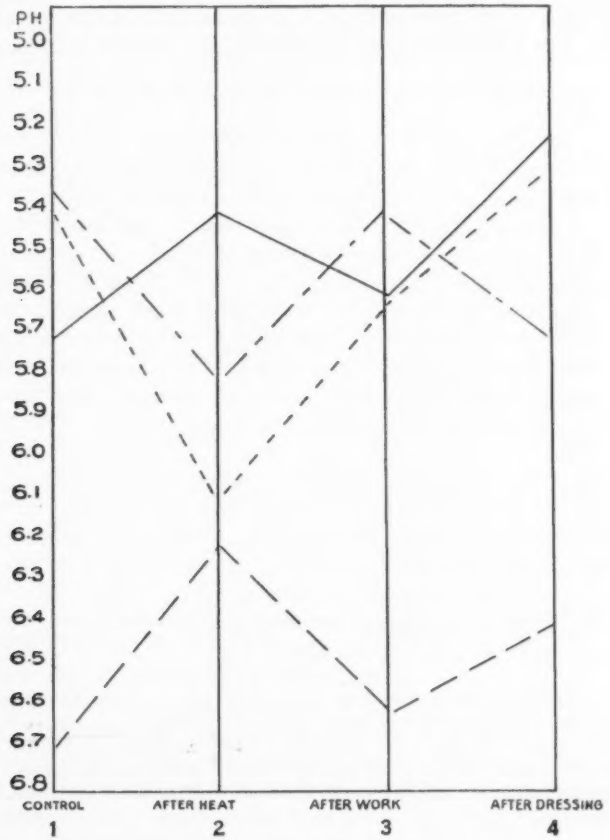

Fig. 1. A chart representing four typical examples of the changes in the hydrogen ion concentrations in urine where work followed immediately after the subjection to heat. Ordinate represents the hydrogen ions in tenths of pH values. Abscissa, the four periods in which urine was taken in 15-minute intervals. Under no. 1, control; under no. 2, after heat; under no. 3, after work; under no. 4, after dressing.

diately after exercise, and the fourth after he had dressed. In this connection it might be stated that the person was subjected to a heat of about 30°C. which rose rapidly to 40° or 45°C.

There were in all eleven experiments in which four samples were taken in the above order. The most remarkable facts shown by these tests were that: *a*, there were different concentrations of the hydrogen ions for each of the four periods; *b*, it seemed impossible to establish anything like a fixed relationship between the periods and the H-ion

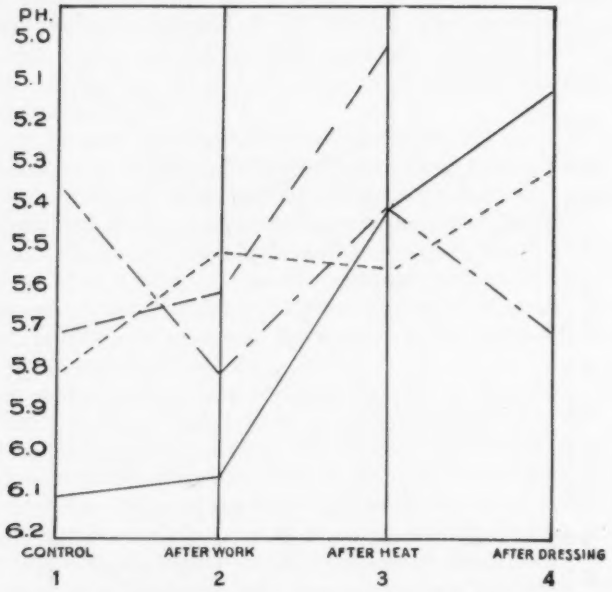

Fig. 2. A chart representing four typical examples of the changes in the hydrogen ion concentrations in urine where heat followed immediately after muscular exercise. Ordinate represents the hydrogen ions in tenth of pH values. Abscissa, the four periods in which urine was taken in 15-minute intervals. Under no. 1, control; under no. 2, after work; under no. 3, after heat; under no. 4, after dressing.

concentration. In most cases the samples obtained in the second, third or fourth period were more acid than was the first sample, which was the control. On the other hand it was most difficult to predict whether the second, third or fourth sample would give the greatest

hydrogen ion concentration. For the sake of clearness I have plotted four typical examples in figure 1, the ordinates representing pH values and the abscissa representing the four samples of urine collected at about fifteen minute intervals.

In the next series of experiments my subjects took the exercise first and then were subjected to heat, which meant that the samples taken at the second and third periods were now reversed. Here again I found just as variable results. In this order ten experiments were performed in which I have plotted four typical cases as shown in figure 2.

These two sets of experiments brought results, as far as urine was concerned, that were difficult to analyze, but yielded some of my most important data on sweat. They seemed to obscure more or less the effects of either work or heat alone upon the urine reaction.

Thereafter the effects of muscular exercise and heat were tested separately. The former will be discussed first. Three samples of urine were now taken: First, just before the exercise, as a control; second, immediately afterwards, and third, after dressing. The results, given in table 1, are listed under subjects G., M. and X. The first two named represent single individuals, while X. represents the report on several individuals. The interval of time between the taking of samples was about twenty minutes.

It is also to be noted that in fourteen experiments with subject G. a greater hydrogen ion concentration was found in the second sample than in the first or control. Two of them show a slight loss while one is unaffected. In comparing the third sample with the control I discovered that twelve out of the fourteen samples had a greater acidity, one sample exhibiting a loss, the other remaining the same. It should also be pointed out that there is only one instance in which the second sample shows the smallest amount of acidity.

In examining the seven experiments performed with subject M., it will be observed that in every instance the urine tested immediately after exercise showed a greater acidity than the control. In the third period four samples are more acid than in the second period, while three show a loss. One of these three is less acid than the control.

In the averages of all the pH values from G., a fairly conspicuous increase in acidity is evident immediately after the exercise, persisting even to the third period, but not so perceptible as the total pH values of 6.04, 5.76 and 5.61, respectively, indicate.

In examining the total averages in the case of M., one observes a difference of 0.3 pH between the first and second periods, with a difference only of 0.03 between the second and third.

Under X. are sixteen experiments in which there were taken only a control and a sample immediately after, corresponding to the second

TABLE 1

SUBJECT	URINE CONTROL	URINE WORK	URINE 20 MINUTES AFTER	SUBJECT	URINE CONTROL	URINE WORK	URINE 20 MINUTES AFTER
	pH	pH	pH		pH	pH	pH
G.	5.65	5.6	5.5	M.	7.0	6.7	5.8
G.	5.5	5.55	5.55	M.	7.4	7.35	7.6
G.	5.9	5.4	5.3	M.	7.5	7.0	7.5
G.	5.9	5.1	5.8	M.	6.9	6.85	6.8
G.	7.0	6.2	5.8	M.	8.5	7.9	7.7
G.	5.6	5.5	5.45	M.	6.8	6.4	6.3
G.	5.9	5.7	5.7	M.	6.7	6.1	6.4
G.	6.0	6.1	6.0				
G.	8.7	7.7	7.0	Av. pH..	7.2	6.9	6.87
G.	5.8	5.7	5.6				
G.	5.75	5.75	5.35	X.	6.0	5.3	
G.	5.8	5.5	5.3	X.	5.2	5.1	
G.	5.7	5.6	5.0	X.	5.6	5.1	
G.	5.4	5.3	5.2	X.	7.4	6.0	
				X.	7.4	6.9	
Av. pH...	6.04	5.76	5.61	X.	6.2	5.9	
				X.	5.7	5.3	
				X.	8.0	4.7	
				X.	7.2	5.3	
				X.	7.2	6.5	
				X.	5.9	5.7	
				X.	6.1	6.15	
				X.	6.0	5.7	
				X.	8.0	5.6	
				X.	5.55	5.5	
				X.	6.2	6.0	
				Av. pH..	6.48	5.67	

period in the other experiments. Of these sixteen determinations, fifteen showed a decided increase in acidity as a result of work, while one shows a decrease of 0.05 pH. The averages here show 6.48 pH for control against 5.67 pH immediately after the exercise. These figures are very significant, and they appear all the more so when one notes the summary of thirty-seven observations as shown in table 2.

In the same manner the effects of heat were studied in order to show how heat may modify the changes of the hydrogen ion of the urine. In table 3 G., M. and X. signify the same subjects as before. In the case of subject G., in fifteen out of eighteen tests the second sample yielded an increase of acidity over the control, in test 2 it showed a slight loss, and in one it remained unaffected. In five instances the second sample was more acid than the third, while in eight cases the third sample was of greater acidity than the second; and in five the acidity was unchanged.

An examination of the results from subject M. show relatively about the same changes. As regards the miscellaneous subjects under X., comparable results were not obtained on account of the fact that the tendency to an increased acidity is delayed to the last period.

TABLE 2

Averages of pH *values as affected by work*

OBSERVATIONS	SUBJECTS	CONTROL	WORK	AFTER
		pH	pH	pH
14	G.	6.04	5.76	5.61
7	M.	7.2	6.9	6.87
16	X.	6.48	5.67	
37	Averages.....	6.57	6.11	6.24

An examination, however, of all the individual tests coupled with the general averages as shown respectively in table 3 and table 4 forces one to the conclusion that heat has a tendency to increase the elimination of acids through the urine. Out of thirty-one observations my results show twenty-nine with an increased acid elimination manifested either in the second or the third sample. The two exceptions are found under X.

These results, then, are quite as striking as those obtained from the effects of exercise and, like the latter, are more significant when one considers the short duration of the experiments and the fact that the subjects were not exposed to excessive heat.

The exact changes that muscular exercise may bring about in the urine by causing an increase of total acidity constitute a question that only further research can settle. It was once thought that, at least as far as normal urines were concerned, the variations of acidity might be explained by the relative proportions of the mono- and di-acid phosphates.

TABLE 3

SUBJECT	URINE CONTROL	URINE HEAT	URINE 20 MINUTES LATER	SUBJECT	URINE CONTROL	URINE HEAT	URINE 20 MINUTES LATER
	pH	pH	pH		pH	pH	pH
G.	5.8	5.9	5.6	M.	6.4	5.8	6.0
G.	5.6	5.45	5.5	M.	5.15	5.05	5.05
G.	5.6	5.5	5.6	M.	5.95	5.9	5.8
G.	5.75	5.5	5.85	M.	6.05	5.25	5.25
G.	5.75	5.7	5.8	M.	7.4	7.4	7.1
G.	6.8	6.0	6.0	M.	8.0	7.6	7.4
G.	5.9	5.8	5.85	M.	6.7	6.1	6.4
G.	6.2	6.0	5.6				
G.	7.6	7.4	7.3	Av. pH..	6.52	6.16	6.14
G.	6.0	5.9	5.9				
G.	5.5	5.55	5.45				
G.	5.6	5.5	5.4	X.	5.7	5.2	5.1
G.	5.7	5.6	5.65	X.	6.2	6.6	6.1
G.	5.4	5.2	5.2	X.	7.5	7.0	5.2
G.	5.8	5.6	5.6	X.	6.2	5.7	5.6
G.	5.75	5.75	5.35	X.	7.1	8.7	8.2
G.	5.8	5.4	5.3	X.	5.8	6.0	6.0
G.	5.7	5.0	5.0				
				Av. pH..	6.42	6.5	6.03
Av. pH...	5.9	5.71	5.66				

TABLE 4

Averages of pH *values as affected by heat*

OBSERVATIONS	SUBJECTS	CONTROL	HEAT	AFTER
		pH	pH	pH
18	G.	5.9	5.71	5.66
7	M.	6.52	6.16	6.14
6	X.	6.42	6.5	6.03
31	Averages.....	6.28	6.12	5.94

Some years ago Folin (9) and also Henderson (10) called attention to the inadequacy of such an explanation. Folin states:

> The current attractive, and in a measure plausible, belief that the acidity of urine is regulated by variations in the relative proportion of the two forms of 'acid phosphates' is therefore erroneous. If urine does at no time contain comparatively strong acids in the free form, the reason is in part the variability of the ammonia formation and in part the presence of salts of organic acid.

Henderson states:

> If phosphoric acid be the chief substance which is varying in amount of base associated with it as the reaction of the urines varies, it is none the less true that other substances are varying too. However, only two, so far as we now know, will at the ordinary physiological ranges show considerable change in combined base. These substances are carbonic acid and uric acid, both of which occur in such small amount as to be of minor importance.

In the examination of Scaffidi's (11) work, which bears directly on the problem, one is led to believe that while the uric acid is normally quite negligible it may become something of a factor as a result of muscular fatigue. While he says that during the exercise there is no increase in uric acid, at the same time he states:

> Immediatement après l'interuption du travail musculaire, il se manifeste une augmentation plus ou moins considérable, mais en tout cas très rapidé et très sensible, dans l'élimination de l'acide urique. Les bases puriniques, dans cette periode diminuent parfois. L'augmentation dans l'élimination de l'acide urique est beaucoup plus sensible quand la fatigue musculaire, et specialement le repos consécutif, ont lieu à des altitudes qui ne sont pas très grandes.

Kenneway (12) has obtained the same results, with this difference: the increase following exercise is only of importance when unaccustomed muscles are brought into use. He claims that after frequent use of these muscles the elimination of uric acid becomes less in amount. Furthermore, he holds that the increased excretion of uric acid immediately following work is not due to the "sweeping out of that which is already present and would otherwise be retained, but that exercise heightened the activity processes by which uric acid is produced," and maintains that the decrease of elimination during work is due to defective oxidation of purine compounds.

Hill and Flack (13) have stated that during severe muscular exercise "the use of oxygen and production of CO_2 are so rapid in the muscles that the circulation cannot keep pace with the demand." Ryffel (14) claimed that the presence of lactic acid in the urine during exercise might be regarded as indicating the inadequacy of the supply of oxygen.

My data are not in harmony with Kenneway's that the continued use of the same set of muscles decreases the elimination of uric acid and that the increase is found only as a result of the use of an unaccustomed set. In my experiments the exercise was done almost entirely on the stationary bicycle, and no depreciation in the acidity resulting from constant use of the same set of muscles.

As to the causes of increased acidity of the urine as a result of heat, we are even more in the dark.

Finally, it should be mentioned that my results conflict with the statement of Vozarik (15) that changes in external temperature, as warm baths, decrease the acidity of the urine.

SUMMARY

1. Intense exercise from fifteen to twenty minutes' duration increases the hydrogen ion concentration of the urine.

2. A man subjected to the heat of a sweat-cabinet for fifteen or twenty minutes will, in a large majority of cases, void a urine of higher hydrogen ion concentration.

3. If muscular exercise follows immediately upon subjection to heat, or vice versa, it is impossible to interpret the results.

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